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ECOLOGICAL STUDIES IN THE LOWER ILLINOIS RIVER VALLEY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 470

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(WITH ONE FIGURE)

Introduction

The central section of Illinois lies between 39° and 41° north latitude. Ecological studies were made adjacent to the Illinois River, in the southwestern part of this section, in Jersey, Calhoun, Greene, and southern Pike counties.

Several factors have contributed to the disturbance of the native flora on the floodplain or bottomland along the Illinois River in the region of this investigation. The two lower dams (10) in the river have raised the water level of the lakes. Discharge of water from the Chicago drainage canal (1, 10) has increased the average water level in this section 2.5–3 feet. Levees (10) have been constructed in many places for the purpose of reclaiming bottom lands for agriculture. The continuity of these levee areas is occasionally interrupted either because of lack of construction or because of the entry of tributaries into the river. Where these gaps occur the land is subject to overflow, and hence is generally not cultivated. These are the only areas representing the prelevee era of the valley, and here the flora has, to a large extent, retained its primitive condition. Such primitive flora was observed at the mouth of Apple Creek.

[1] Research paper no. 368, Journal Series, University of Arkansas.

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Physiography and geology

The Illinois River (**2, 9**), formed by the junction of the Des Plaines and Kankakee rivers in northeastern Illinois, flows 273 miles across the state in a southwesterly direction and joins the Mississippi River at Grafton. The fall of the river from LaSalle to its mouth, a distance of 223 miles, is only 33 feet (**1**). Its floodplain or bottomland is bounded by bluffs or hills, and it varies from 1.5 to 7 miles in width, averaging about 4 miles within the range of this study.

The bottomland of the lower valley of the Illinois River has been filled to a greater depth than its upper valley (**1**). Here the immediate banks of the stream are high, and although the lakes are smaller and fewer than in the upper valley, many of them lie 10 feet or more above the river when the water is low.

The lakes of the floodplain are not of the oxbow type. They have been formed by the impounding of water brought about by the differential deposition of silt, and by the formation of natural levees. These lakes lie, for the most part, in the lower backwater areas of the floodplain. They occur both in the forest and in the wet prairie. The lakes have been classified as permanent and temporary. Where there are smaller exposed surfaces of water these areas are called ponds or sloughs. The lakes receive their water supply from springs or small tributaries to the river. The permanent lakes are deep and poorly drained. The temporary lakes are made shallow by frequent deposits of silt. They become dry during part of the year, except in extremely wet years. During the exceedingly dry year of 1931 some of the "permanent" lakes also became dry.

In the region studied, the rocks (**9**) immediately underlying the upper soil layers of loess and loam are Mississippian limestone, containing some cherty and oolitic material and overlying shale of the same epoch. The bluffs at the sides of the river valley, throughout the area, have this same composition, with the exception of southern Calhoun County where there are small outcrops of Devonian and Silurian limestone overlying thicker beds of Ordovician (Richmond) limestone and shale. The section west of the Illinois River comprising Calhoun County and the southernmost part of Pike County were unglaciated (**7, 8**).

Ecological factors

Climate

The physiographic features of central Illinois have little effect upon its climate (**14**). This section lies in the path of the main storm tracks that cross the country. Especially in winter these cause marked changes in the weather. The climatological data here given are from weather bureau stations in central Illinois to 1930, inclusive.

Temperature.—Central Illinois has a mean annual temperature of 52.6° F. The mean annual temperature in January, the coldest month, is 27.0°; in July, the warmest month, 76.4°. "The average minimum temperature for January is 18.4°; the average minimum temperature for July is 88.5°" (**13**). The crop-growing season averages 178 days.

Precipitation.—The average annual rainfall is 36.65 inches with an annual range of 32.83 to 40.74 inches. Summer rainfall is sufficient to meet the requirement of the vegetation, although occasional droughts may occur. Most of the rainfall is of the local shower type. The snowfall averages 20.3 inches annually.

Air movement.—The prevailing winds in winter are from the northwest; during the summer they are from a southerly direction. Over limited areas occur occasional local squalls, and sometimes tornadoes, which cause great destruction.

Soils

The present soils were derived from the residual material of the unglaciated area, glacial drift, and loess or windblown glacial material. Soils and their formation were studied particularly in Pike (**7**) and Calhoun (**8**) counties. Three topographical areas, each with its soil types, occur in this region: the upland, the bluff, and the bottomland.

upland

The upland is characterized by two major soils, the upland prairie and the upland timber.

Upland prairie soils.—Because of the large quantity of roots of prairie grasses in the upland prairie soils, they are the richest in organic matter of all the soils in this region. These soil areas are rather

limited in extent. There is evidence that trees have recently grown on small areas of the prairie. Four types of prairie soils are found. The brown silt loam, although occupying little territory, has fair drainage and is the most extensive and fertile of the upland soils. Here is found the best assemblage of relic prairie plants. The sticky black clay loam (gumbo) is found on the poorly drained areas. The variation in content of organic matter indicates that the brown-gray silt loam on tight clay may have been timbered. It usually occurs between the timbered areas and the brown silt loam. The gray silt loam on tight clay occurs on level to slightly undulating topography. Because of the tight clay subsoil, it has poor drainage. It also contains the least organic matter of all the prairie soils.

UPLAND TIMBER SOILS.—The upland timber soils have a lower content of organic matter than the prairie soils. Yellow silt loam and yellow-gray silt loam, both derived from loess, are the principal upland timber soil types inland in Pike County (**7**). They are found on the somewhat level ridges of the rolling hills and on places badly broken by erosion. These broken areas are covered by plants of the oak-hickory association.

On the flat tops of the ridges and occupying only a small area is a light gray silt loam which is friable and porous. A layer of clay which is almost impervious to both air and water underlies this soil. This is a poorly drained but not a swampy soil.

The upland adjoining the Illinois River consists of a yellow-gray fine sandy loam on the less rolling land, and a yellow fine sandy loam on the hilly and very rolling, badly broken land of the deep loess areas. It is very pervious and hence has good drainage. The coarse sandy phases of this soil are found nearest the bluff.

The Calhoun County soil report (**8**) refers to the two soils just mentioned for Pike County as brownish yellow-gray silt loam and eroded silt loam. These are the only upland soils occurring in Calhoun County. The brownish yellow-gray silt loam increases in area toward the southeastern end of the county. All of the upland soils of this county were developed under a forest cover. They are derived from loess which overlies rock in most of the county. Almost no soil profile has been developed. It is fairly well developed only in the brownish yellow-gray silt loam. Active erosion has prevented the

development of a true soil profile in most of the county. These soils are friable and have no impervious strata in their profile; hence they have good drainage. Upon the uncultivated areas of this soil were oak, hickories, and white ash.

FLOODPLAIN OR BOTTOMLAND

BOTTOMLAND SOILS.—The valleys of the tributaries and small areas of bottomland of the Illinois River contain some soil deposits derived from the older glaciations. Most of the bottomland soils are of recent origin, however, and show no profile development. This is accounted for by annual recurrence of floods.

Floods (**15**) covering the floodplain to depths of 5 to 15 feet were recorded in 1883, 1892, 1893, 1903, 1904, 1908, 1913, 1916, 1920, 1922, 1926, 1927, and 1929 in the region near Kampsville. Minor floods submerging the bottoms to a depth of 1 to 5 feet occur almost annually. Most floods follow melting of the ice and snow in spring. Occasional floods occur in midsummer or fall. The spring floods usually come before the foliating period and seldom have any injurious effect except that caused by alluvial deposition.

The bottomland soils of the Illinois River seem to have three primary modes of origin: soils brought down from the upland by tributaries; coarse soils deposited by overflow of the river at flood time; and soils composed of the fine material that has settled in the backwater areas at the time of overflow. The following three soil types derived from these modes of origin have been recognized.

1. Soils carried down from the uplands are deposited as alluvial fans at the mouth of the tributaries. Brownish yellow-gray silt loam deposited upon the terraces at the base of the bluffs overlies sand and gravel. It is made up of a mixture of limestone fragments, hillside wash, and a small amount of river-deposited alluvium. It is a well drained soil and subject to overflow only in times of extremely high flood water. This soil type is always populated by the talus slope-floodplain transition forest and some members of the hillside talus slope and floodplain forests. Other deposits made near the mouths of the tributaries become a brown silt loam. This is covered either by forest or by floodplain prairie association.

2. The remaining soils are those derived primarily from the soils

deposited by the Illinois River. Mixed loam is the prevailing soil type in the belt bordering the river channel and on the islands. It consists of the coarsest materials deposited by the flood water. Typical floodplain forest grows upon this soil. Upon the rock or coarse soil of the islands a soil 1 to 5–6 feet in depth has developed. On slightly higher land is a deep brown clay loam containing some coarser material. There is evidence that the lower lying parts of this area were once populated rather extensively by the *Spartina michauxiana-Panicum virgatum* association. Most of this soil is now under cultivation. On the higher parts was found the floodplain forest.

3. In the low-lying, nearly level backwater areas, where lakes, ponds, and sloughs are commonly found, the soil is a drab clay loam. This has the finest texture and is the heaviest of all bottomland soils. It has poor drainage. Floodplain forest seldom occurs on this soil, but there is found typically the *Cephalanthus-Hibiscus militaris* association with occasional willows and green ash. Sometimes *Eleocharis* dominates this habitat, and more rare is the occurrence of the *Spartina michauxiana-Panicum virgatum* association.

BLUFF

BLUFF FACE.—The perpendicular face of the limestone bluffs provides a habitat for very few plant species. Residual and windblown soils accumulate in rock crevices where only a few herbaceous seed plants can grow.

TALUS SLOPE.—The talus slopes at the base of the bluffs consist of fragments of limestone and chert of varying size covered by residual soil and the brownish yellow silt loam washed down from the upland. Upon the surface is a rich mulch of partially decayed organic matter which, as in the case of the upland soils, does not combine to a great extent with the underlying soils. Such trees as red and hard maples, red and chestnut oaks, walnut and butternut, and basswood grow on these slopes.

HYDROGEN-ION CONCENTRATION

Soil samples for the determination of pH were taken at a depth of 10 inches. The upland soils had a hydrogen-ion concentration which ranged from neutral to pH 6. In both the upland forest and the prairie, pH 6.6 was common. The lowest pH values were found in

old fields in which there had been repeated cropping and excessive leaching.

While the floodplain soils are in general circumneutral, in this region the pH ranged from 7 to 7.8, indicating a more alkaline soil. This corresponds with the pH of the yearly flood water of the river, which has consistently tested 7.8. Floodplain lakes, ponds, and ditches also had a pH of approximately 7.8. The pH of the talus slope soil was 8.

Floristics

A transect of the Illinois River valley in the region investigated shows four sections: the upland, the bluff, the transition, and the floodplain. Each of these sections was divided into plant communities.

The plants[2] observed and recorded are divided into three groups: (1) large and small trees, and shrubs of greatest frequence, studied quantitatively and enumerated in the tables; (2) woody plants of infrequent occurrence; and (3) all herbs and some woody plants occurring in spring, summer, and fall aspects. The third group of plants has been assembled in three classes: class 1, the most common plants and those frequently occupying relatively large areas; class 2, the plants occurring fairly commonly but rarely occupying areas of wide extent; class 3, plants occurring occasionally. The order of arrangement for all species is from that of greatest to that of least frequence. Casual or rare plants are not listed.

Plant communities

Upland

Upland pastures and old fields.—Because of the fragmentary occurrence and difficulty in identifying the upland prairie vegetation among the upland pastures and old fields, neither a quantitative evaluation nor a study of dominance and succession was made in this community. The data assembled, however, are of value because they give a partial record of the present condition of a plant community that is rapidly disappearing.

[2] Authorities for the species listed are those given in the seventh edition of Gray's Manual. The species of Gramineae were identified by Mrs. Agnes Chase of the United States Department of Agriculture. Approximately 650 species of plants were collected, and a set was given to the Department of Botany at the University of Chicago.

HERBS

Spring

Class 1

Poa pratensis
Trifolium repens
Rumex acetosella
Capsella bursa-pastoris
Erigeron pulchellus
E. philadelphicus
Potentilla canadensis
Poa annua
P. compressa

Class 2

Melilotus alba
M. officinalis
Stellaria media
Antennaria plantaginifolia
Medicago sativa
Plantago lanceolata
P. major
Oxalis stricta
Apocynum cannabinum
Achillea millefolium
Verbascum blattaria

Class 3

Pentstemon gracilis
Asclepias quadrifolia
Silene antirrhina
Physalis virginiana
Arabis drummondi
Fragaria virginiana
Amorpha canescens
Phlox divaricata
Comandra umbellata
Monarda bradburiana
Blephilia hirsuta
Polygonatum commutatum
Scutellaria versicolor

Summer

Class 1

Erigeron spp.
Ipomoea hederacea
Verbena stricta
Euphorbia corollata
Verbascum thapsus
Strophostyles helvola
Stellaria media
Rudbeckia hirta
Plantago aristata
Petalostemum purpureum
Cirsium lanceolatum
Silphium integrifolium

Class 2

Agrostis hyemalis	Desmodium illinoense
Hypericum punctatum	Monarda fistulosa
Bouteloua curtipendula	Ambrosia bidentata
Solidago radula	Phleum pratense
Sorghum halapense	Asclepias tuberosa

Class 3

Daucus carota	Nepeta cataria
Solanum carolinense	Verbena bracteosa
S. nigrum	Achillea millefolium
Asclepias purpurascens	Croton capitatus
Lechea tenuifolia	Physalis heterophylla
Oxalis corniculata	Psoralea tenuiflora
Ruellia ciliosa	Kuhnia eupatorioides
Verbena urticaefolia	Cichorium intybus
Petalostemum candidum	Acerates floridana
Setaria glauca	Pycnanthemum muticum
Coreopsis tripteris	Tripsacum dactyloides
Astragalus canadensis	Cuphea petiolata
Phlox pilosa	Panicum capillare
Anthemis cotula	Pentstemon grandiflorus

Fall

Class 1

Eupatorium torreyanum	Panicum agrostoides
E. sessilifolium	Sporobolus asper
Andropogon furcatus	Andropogon scoparius
Eragrostis cilianensis	Helianthus hirsutus

Class 2

Gerardia tenuifolia	Aster oblongifolius
Aster anomalus	Cirsium arvense
Eragrostis caroliniana	Lespedeza capitata
Liatris cylindracea	Sorghastrum nutans
L. scariosa	Solidago spp.
Cassia nictitans	

Class 3

Croton monanthogynus	L. virginica
Polanisia trachysperma	L. frutescens
Lespedeza repens	Elymus canadensis
L. striata	Liatris pycnostachya

SHRUBS, PERMANENT INVADERS OF OPEN AREAS

Rhus toxicodendron	Symphoricarpos orbiculatus
R. canadensis	
R. glabra	Corylus americana
Cornus asperifolia	Sassafras variifolium
Sambucus canadensis	Rosa cinnamomea

At the beginning of spring in this prairie region a considerable number of introduced species appear, many of which are common weeds. As the season progresses, however, the aspect of the vegetation changes and by midsummer there are many typical prairie species. Most of these are apparently survivors of the previously widespread prairie of the central states. Several are western species, some of which are at the eastern borders of their range; others are probably extraneous.

UPLAND FOREST.—The transect method was used in making a quantitative study of the woody plants in the various plant communities. Each quadrat was 50 meters in length and 10 meters in width, and fifty quadrats were studied in each plant community. A record was made only of trees 6 inches or more in diameter, breast high, and of shrubs and small trees 6 feet or more in height. The data in the tables list the species and give: (1) the number of quadrats, in the 50 quadrats, in which each species is found; (2) the average number of individuals in a quadrat, computed on a 50-quadrat basis; and (3) the dominance index. The dominance index was obtained by multiplying the average number of quadrats in which a species was found by the average number of individuals of that species in a quadrat.

In the upland forest (table I) the 50 quadrats were distributed over 20 miles of upland bordering the Illinois River. Most of the

woodlands of the region have been cut over, burned, and grazed; hence their resemblance to the original stand is questioned. From the size and apparent age of the tr one may conclude that some of them in a few of the timber tra persist from the virgin stand.

TABLE I

QUANTITATIVE EVALUATION OF TREES AND SHRUBS OF GREATEST FREQUENCE IN UPLAND FOREST

SPECIES	NO. QUADRATS CONTAINING SPECIES	AVERAGE NO. INDIVIDUALS PER QUADRAT	DOMINANCE INDEX
Trees			
Quercus alba	50	10.0	500
Q. velutina	50	4.2	210
Q. rubra	50	3.5	175
Carya alba	47	3.3	155
C. ovata	38	4.0	152
Acer saccharum	20	2.7	54
Fraxinus americana	23	1.8	41
Acer rubrum	14	2.3	32
Juglans nigra	19	1.6	30
Quercus muhlenbergii	5	1.6	8
Morus rubra	5	1.0	5
Smaller trees and shrubs*			
Cercis canadensis	16	4.0	64
Cornus florida	25	2.5	61
Sassafras variifolium	11	4.0	44
Rhus glabra	3	8.0	24
Viburnum rufidulum	7	3.0	21
Cornus asperifolia	8	1.0	8

* *Ceanothus americanus* is the most common and widely distributed of the shrubs but was not recorded because of its small size.

The tree species as recorded by the dominance index, together with the list of woody and herbaceous plants, indicate that the upland forest is a typical oak-hickory forest association.

TREES OF INFREQUENT OCCURRENCE

Diospyros virginiana
Aesculus glabra
Prunus serotina
P. americana
Juniperus virginiana
Ulmus americana
U. fulva
Juglans cinerea
Quercus macrocarpa

SMALL TREES AND SHRUBS OF INFREQUENT OCCURRENCE

Rhus canadensis
Rubus recurvans
R. villosus
Hydrangea arborescens
Ribes gracile
Crataegus spp.
Evonymus atropurpureus
Amelanchier canadensis
Pyrus ioensis
Zanthoxylum americanum
Rosa setigera
Viburnum prunifolium
Ptelea trifoliata
Staphylea trifolia
Rhamnus lanceolata

VINES

Rhus toxicodendron
Psedera quinquefolia
Smilax rotundifolia
S. hispida
Celastrus scandens
Vitis aestivalis
Tecoma radicans

HERBS

Spring

Class 1

Claytonia virginica
Phlox divaricata
Podophyllum peltatum
Galium spp.
Phlox pilosa
Dicentra cucullaria
Ranunculus hispidus
Trillium recurvatum
Dicentra canadensis
Viola sororia
Erythronium albidum

Class 2

Asarum canadense
Triosteum aurantiacum
Antennaria plantaginifolia
Erigeron pulchellus
Potentilla canadensis
Monarda bradburiana
Sanicula gregaria
Uvularia grandiflora
Comandra umbellata
Hepatica triloba
Camassia esculenta
Nothoscordum bivalve
Uvularia perfoliata

Class 3

Lithospermum canescens
Hybanthus concolor
Oxalis stricta
Asclepias quadrifolia
Sphenopholis obtusata
Brauneria pallida
Apocynum cannabinum var. pubescens
Poa pratensis
Actaea alba
Oxalis violacea
Pentstemon gracilis
Polygonatum commutatum
Physalis subglabrata
Scutellaria versicolor
Allium canadense
Castilleja coccinea
Dodecatheon meadia
Actaea rubra
Viola rafinesquii

Summer

Class 1

Amorpha canescens
Galium circaezans
G. pilosum
Cuscuta cuspidata
Euphorbia corollata
Cuscuta coryli
Gillenia stipulata

Class 2

Monarda fistulosa
M. punctata
Gerardia grandiflora
Pycnanthemum flexuosum
Physalis pubescens
Psoralea onobrychis
Anemone virginiana
Petalostemum purpureum

Class 3

Asclepias purpurascens
Oxalis corniculata
Asclepias verticillata
Petalostemum candidum
Ruellia ciliosa
Geum canadense
Hystrix patula
Eupatorium urticaefolium
Eryngium yuccifolium
Lilium philadelphicum

Fall

Class 1

Eupatorium torreyanum
Desmodium sessilifolium
Solidago spp.
Helianthus hirsutus

Class 2

Gerardia tenuifolia	Baptisia leucantha
Vernonia altissima	Aster spp.

Class 3

Cassia nictitans	L. squarrosa
Liatris cylindracea	L. scariosa

BLUFF

LIMESTONE BLUFF.—There are two groups of plants commonly found in the limestone bluff habitat, those growing upon the bare rock or in crevices containing little or no soil and those living in crevices or depressions having considerable soil. All divisions of the plant kingdom occurred among the first group of plants. The lichens and mosses were unidentified. Two genera of ferns were named, *Pellaea atropurpurea* and *Cheilanthes feei*. Although usually requiring more soil, *Heuchera americana*, *Houstonia* sp., and *Elymus canadensis* were the seed plants found in this group.

The second plant group here listed is larger, and it is more variable in its soil requirements than is the first plant group. Among the species listed are those which are also found on the hilltop and in the talus slope forest.

WOODY PLANTS

Class 1

Psedera quinquefolia	Celastrus scandens
Rhus toxicodendron	Vitis aestivalis

Class 2

Hydrangea arborescens	Staphylea trifolia
Lonicera sempervirens	Juniperus virginiana
Rhus canadensis	Cercis canadensis
Ptelea trifoliata	Cornus asperifolia

Class 3

Rhamnus lanceolata	Ceanothus americanus
Viburnum prunifolium	Gymnocladus dioica
Amelanchier canadensis	

HERBS

Spring

Class 1

Aquilegia canadensis
Houstonia lanceolata
Heuchera americana
Pellaea atropurpurea
Cheilanthes feei

Class 2

Triosteum aurantiacum
Comandra umbellata
Phlox pilosa

Class 3

Oxalis violacea
Dodecatheon meadia
Oxalis stricta
Monarda bradburiana
Pentstemon gracilis
Polygonatum commutatum

Summer

Class 1

Houstonia lanceolata

Class 2

Campanula americana

Fall

Class 2

Elymus canadensis
Solidago spp.

The spring flora in this plant community consists of but few species. These, however, constitute the largest number of herbaceous plants. The number of new species and of persisting plants diminishes throughout the summer, and by September the herbaceous vegetation has almost entirely disappeared.

TRANSITION

HILLSIDE-TALUS SLOPE FOREST.—Between the floodplain terraces and the bluffs there are steep slopes upon which grow the hillside-talus slope forest. The quantitative analysis of the woody vegetation of this forest is given in tables II and III.

Owing to the cutting of the red, white, and black oaks for use as timber and firewood, the dominance index of the tree species as given in table II probably does not give a picture of the virgin forest that

TABLE II

QUANTITATIVE EVALUATION OF TREES OF GREATEST FREQUENCE IN HILLSIDE-TALUS SLOPE FOREST

SPECIES	NO. QUADRATS CONTAINING SPECIES	AVERAGE NO. INDIVIDUALS PER QUADRAT	DOMINANCE INDEX
Acer saccharum	50	10.2	510
Quercus muhlenbergii	48	7.1	341
Q. rubra	36	7.1	266
Tilia americana	46	3.7	170
Juglans nigra	42	3.5	147
Gymnocladus dioica	27	3.7	100
Carya ovata	30	2.5	75
C. alba	33	2.0	66
Acer rubrum	31	2.1	65
Ulmus americana	26	2.4	62
Aesculus glabra	20	2.3	46
Juglans cinerea	20	2.1	42
Celtis occidentalis	21	2.0	42
Ulmus fulva	25	1.5	38
Quercus velutina	8	4.0	32
Robinia pseudo-acacia	9	2.3	21
Fraxinus americana	17	1.2	20
Carya glabra	10	1.4	14

TABLE III

QUANTITATIVE EVALUATION OF SMALL TREES AND SHRUBS OF GREATEST FREQUENCE IN THE HILLSIDE-TALUS SLOPE FOREST

SPECIES*	NO. QUADRATS CONTAINING SPECIES	AVERAGE NO. INDIVIDUALS PER QUADRAT	DOMINANCE INDEX
Cercis canadensis	37	4.7	174
Staphylea trifolia	28	3.8	106
Cornus asperifolia	30	3.3	99
Ptelea trifoliata	21	4.7	99
Asimina triloba	16	2.8	45
Cornus florida	20	2.0	40
Sassafras variifolium	14	2.0	28
Rhus glabra	13	2.0	26
Diospyros virginiana	8	1.6	13
Carpinus caroliniana	5	2.0	10
Ostrya virginiana	4	2.0	8

* *Hydrangea arborescens* was not recorded because of its small size, although it is one of the most abundant and widespread shrubs in this forest.

occupied this plant community. Lists of the infrequent woody plants and of the herbs in the undergrowth are included in this study. A larger number of woody species, and possibly as many, if not more, herbaceous species are found here than in any other habitat in this region.

TREES OF INFREQUENT OCCURRENCE

Prunus serotina
Quercus alba
Morus rubra
Acer negundo
Quercus macrocarpa
Q. imbricaria
Fraxinus pennsylvanica var. lanceolata
Carya illinoensis
C. cordiformis
Platanus occidentalis
Gleditsia triacanthos
Populus alba

SMALL TREES AND SHRUBS OF INFREQUENT OCCURRENCE

Rubus spp.
Sambucus canadensis
Viburnum rufidulum
Amelanchier canadensis
Juniperus virginiana
Crataegus spp.
Benzoin aestivale
Ribes gracile
Rhamnus lanceolata

VINES

Rhus toxicodendron
Psedera quinquefolia
Menispermum canadense
Vitis aestivalis
Cissus ampelopsis
Vitis vulpina
Smilax rotundifolia
Humulus lupulus
Celastrus scandens
Clematis pitcheri

HERBS

Spring

Class 1

Claytonia virginica
Ranunculus hispidus
Viola sororia
Podophyllum peltatum
Phlox divaricata
Aquilegia canadensis
Trillium recurvatum
Polystichum acrostichoides
Geranium maculatum
Adiantum pedatum
Arisaema triphyllum
Delphinium tricorne

Dicentra cucullaria
Galium pilosum
Asarum canadense
Anemonella thalictroides
Onoclea sensibilis
Cystopteris fragilis

Class 2

Smilacina racemosa
Dicentra canadensis
Polygonatum commutatum
Sanguinaria canadensis
Anemone canadensis
Menispermum canadense
Geranium carolinianum
Botrychium virginianum
Osmorhiza claytoni
Sanicula gregaria
Cerastium arvense var. oblongifolium
Uvularia grandiflora
Dentaria laciniata
Triosteum aurantiacum
Caulophyllum thalictroides
Viola pubescens
Aruncus sylvester
Mertensia virginica
Hydrophyllum virginianum
Asplenium acrostichoides

Class 3

Ruellia strepens
Hepatica triloba
Hybanthus concolor
Osmorhiza longistylis
Smilax herbacea
Monarda bradburiana
Panax quinquefolium
Pedicularis canadensis
Trillium grandiflorum
Arabis laevigata
Oxybaphus nyctagineus
Heuchera americana
Cypripedium parviflorum
Sphenopholis obtusata
Liparis liliifolia
Hydrastis canadensis
Actaea rubra

Summer

Class 1

Podophyllum peltatum
Phlox divaricata
Galium spp.
Adiantum pedatum
Aquilegia canadensis
Polystichum acrostichoides
Desmodium bracteosum
Impatiens biflora
I. pallida
Onoclea sensibilis
Cystopteris fragilis

Class 2

Cuscuta coryli
Aralia racemosa
Botrychium virginianum
Campanula americana
Eupatorium purpureum
Cryptotaenia canadensis
Asplenium acrostichoides
Anemone virginiana
Phlox paniculata
Tradescantia reflexa
Dioscorea villosa
Hydrophyllum virginianum
Polymnia canadensis
Aruncus sylvester
Scrophularia marilandica
Phytolacca decandra

Class 3

Eupatorium urticaefolium
Aspidium marginale
Geum canadense
Circaea lutetiana
Lobelia siphilitica
Hystrix patula
Heliopsis scabra
Gerardia flava
Gillenia stipulata
Actinomeris alternifolia
Sicyos angulatus
Lilium canadense
Scutellaria pilosa
Panax quinquefolium
Hydrastis canadensis
Rudbeckia triloba
Cystopteris bulbifera
Polymnia canadensis var. radiata

Fall

Class 1

Eupatorium serotinum
Kuhnia eupatorioides
Gnaphalium polycephalum
Desmodium sessilifolium

Class 2

Setaria glauca
Cirsium lanceolatum
Aster spp.
Conobea multifida
Actinomeris alternifolia

Class 3

Eupatorium sessilifolium
Brauneria purpurea
Panicum praecocius
Anychia canadensis
Eupatorium urticaefolium
Cassia nictitans
Digitaria sanguinalis
Cirsium discolor
Panicum clandestinum
Muhlenbergia sobolifera
Brachyelytrum erectum
Eragrostis capillaris

TALUS SLOPE-FLOODPLAIN TRANSITION FOREST.—The floodplain terrace, which lies between the talus slope and the flat bottomland, slopes gently toward the river, and upon it grows the talus slope-floodplain transition forest.

In this transition zone is found a mixture of forest trees (table IV). The American elm, burr oak, pecan, sycamore, hackberry, green ash, honey locust, and soft maple are representative floodplain trees; while walnut, butternut, red bud, white ash, and Kentucky coffee

TABLE IV

QUANTITATIVE EVALUATION OF TREES OF GREATEST FREQUENCE IN TALUS SLOPE-FLOODPLAIN TRANSITION FOREST

SPECIES	NO. QUADRATS CONTAINING SPECIES	AVERAGE NO. INDIVIDUALS PER QUADRAT	DOMINANCE INDEX
Ulmus americana	48	10	480
Platanus occidentalis	50	6.5	325
Celtis occidentalis	40	5	200
Cercis canadensis	33	3	99
Acer saccharinum	36	3.2	85
Carya illinoensis	27	2.8	76
Acer negundo	23	3	69
Quercus macrocarpa	27	2.3	62
Gleditsia triacanthos	21	1.8	58
Cornus asperifolia	17	3	51
Gymnocladus dioica	22	2	44
Diospyros virginiana	18	2	36
Fraxinus americana	16	2	32
Juglans nigra	18	1.3	23
Tilia americana	12	1.3	16
Juglans cinerea	8	1.5	12

bean are typical hillside-talus slope trees. Significant is the fact that the American elm, sycamore, burr oak, box elder, and hackberry attain their maximum concentration or dominance in this habitat.

TREES AND SHRUBS OF INFREQUENT OCCURRENCE

Crataegus spp.
Carya cordiformis
Quercus muhlenbergii
Adelia acuminata
Salix longifolia
Ulmus fulva
Prunus serotina
Asimina triloba
Populus deltoides
Sassafras variifolium
Sambucus canadensis

VINES

Psedera quinquefolia
Rhus toxicodendron
Vitis vulpina
Smilax rotundifolia
Smilax hispida
Tecoma radicans
Menispermum canadense
Cissus ampelopsis

HERBS

Spring

Class 1

Claytonia virginica
Phlox divaricata
Podophyllum peltatum
Ranunculus septentrionalis
Delphinium tricorne
Trillium recurvatum
Ranunculus abortivus
Capsella bursa-pastoris
Cerastium arvense var. oblongifolium
Mertensia virginica
Anemonella thalictroides
Viola sororia
Anemone canadensis
Arisaema triphyllum
Geranium carolinianum
Ranunculus hispidus
Smilacina racemosa
Hydrophyllum virginianum
Arisaema dracontium
Amsonia tabernaemontana
Potentilla monspeliensis
Veronica officinalis
Arabis virginica
Veronica peregrina
Chaerophyllum procumbens
Rumex acetosella
Viola cucullata
Osmorhiza claytoni
O. longistylis

Class 2

Solanum triflorum
Cerastium vulgatum
Specularia perfoliata
Convolvulus sepium
Viola rafinesquii
Oxybaphus nyctagineus
Senecio integerrimus
Botrychium virginianum
Apocynum cannabinum var. pubescens
Ruellia strepens

Class 3

Lythrum alatum
Smilax herbacea
Plantago lanceolata
Pedicularis canadensis

Bromus secalinus
Plantago virginica
Marrubium vulgare
Achillea millefolium
Malva rotundifolia
Arabis patens
Amorpha fruticosa
Festuca elatior

Summer

Class 1

Sicyos angulatus
Campanula americana
Tradescantia pilosa
Cassia chamaecrista var. robusta

Class 2

Impatiens biflora
Ruellia ciliosa
Desmodium bracteosum
Clematis pitcheri
Veronica virginica
Eupatorium purpureum
Phytolacca decandra

Class 3

Prunella vulgaris
Silene stellata
Gerardia flava
Leersia virginica
Cacalia reniformis
Aspidium marginale
Polygonum setaceum
Silphium perfoliatum
Saponaria officinalis
Rudbeckia laciniata
Lobelia inflata
Anemone virginiana
Hystrix patula
Verbascum thapsus
Nepeta cataria

Fall

Class 1

Eupatorium serotinum
Solidago serotina
Heliopsis scabra
Chenopodium glaucum

Class 2

Eupatorium perfoliatum
Rudbeckia triloba
Humulus lupulus
Cuscuta glomerata

Class 3

Eupatorium urticaefolium
Conobea multifida
Panicum clandestinum
Muhlenbergia sobolifera

Brachyelytrum erectum
Croton glandulosus var. septentrionalis
Euphorbia dentata
Sorghum halapense

FLOODPLAIN

The floodplain or bottomland includes the remaining lowland vegetation between the terrace and the channel of the Illinois River. This topographic unit has provided the best opportunity for the study of the hydrarch plant succession, which may be seen in forest, prairie, lake, river bank, and island plant communities.

TABLE V

QUANTITATIVE EVALUATION OF TREES OF GREATEST FREQUENCE IN THE FLOODPLAIN FOREST

Species	No. quadrats containing species	Average no. individuals per quadrat	Dominance index
Acer saccharinum	48	8.7	418
Ulmus americana	50	6.4	320
Carya illinoensis	47	4.1	193
Quercus palustris	45	3.4	153
Adelia acuminata	36	3.7	133
Crataegus sp.	36	3.2	115
Celtis occidentalis	38	3.0	114
Fraxinus pennsylvanica var. lanceolata	42	2.6	109
Betula nigra	23	3.0	69
Gleditsia triacanthos	16	2.0	32
Diospyros virginiana	16	1.6	26
Populus deltoides	12	2.0	24

Floodplain forest.—The habitat of the floodplain forest varied from the moister area bordering swamps, sloughs, or river bank to the higher, drier parts of the floodplain.

The dominance index of the floodplain forest (table V) shows that the soft maple and American elm are the dominant trees; pecan, hackberry, pin oak, swamp privet, and *Crataegus* are second in abundance; while river birch, honey locust, persimmon, and cottonwood occur in lesser abundance. The cottonwood, occurring most abundantly in moist or pioneer areas, was the pioneer tree of the floodplain forest. It persisted in the early stages of floodplain succession but was rarely found in areas where the climax or subclimax forest was established.

The pecan and pin oak were occasionally important, forming pure stands locally. The cutting of less desirable trees may account for this dominance of the pecan. In a few limited areas, north of the region studied, the pin oak occupied 40 per cent of the timber stand.

In early spring in the lower, wetter parts of the floodplain forest occur *Senecio glabellus* and *Radicula palustris*, and colonies of *Amsonia tabernaemontana*, *Erigeron*, and *Apocynum cannabinum*. The higher, better drained parts of this forest contain several species of *Ranunculus* (*R. septentrionalis* being the most common), *Phlox divaricata*, *Anemone* spp., *Podophyllum*, *Claytonia*, *Trillium*, *Geranium*, and *Viola* spp. Summer finds an abundance of *Polygonum*, *Steironema*, *Stachys*, *Chenopodium*, *Lysimachia*, and *Teucrium*. Species of these genera may be intermingled, or any one of them may form societies of varied extent. In late summer and fall composites dominate the habitat, and *Eupatorium serotinum* is the most abundant species. *Vernonia illinoensis* and *V. missurica* are conspicuous and important, and commonly form extensive societies in the more open lowland forest. In damp, well shaded parts of the forest, *Laportea* is frequently dominant, and associated with it as a subdominant may be *Impatiens biflora*. In September *Boltonia asteroides* is common in the more open areas, *Vernonia* spp. persist, *Matricaria suaveolens* appears, and *Physostegia virginiana* occurs along the forest edge. Species of *Solidago* and *Bidens* occur sparingly in the floodplain forest but are abundant in the adjacent floodplain prairie and abandoned fields.

TREES OF INFREQUENT OCCURRENCE

Platanus occidentalis	Ulmus fulva
Carya laciniosa	Gleditsia aquatica
Morus rubra	Quercus lyrata
Quercus bicolor	

VINES

Smilax rotundifolia	Psedera quinquefolia
S. hispida	Rhus toxicodendron
Menispermum canadense	Tecoma radicans
Vitis vulpina	Cissus ampelopsis
V. cordifolia	

HERBS

Spring

Class 1

Radicula palustris
Senecio glabellus
Phlox divaricata
Ranunculus septentrionalis
Potentilla monspeliensis
Claytonia virginica
Podophyllum peltatum
Arisaema triphyllum
Geranium maculatum
Anemone canadensis
Trillium recurvatum
Hydrophyllum virginianum
Arisaema dracontium

Class 2

Ranunculus abortivus
Chaerophyllum procumbens
Erigeron pulchellus
E. philadelphicus
Galium triflorum
Apocynum cannabinum var. pubescens
Amsonia tabernaemontana
Viola cucullata
V. pubescens
V. missouriensis

Class 3

Spermacoce glabra
Iris versicolor
Ranunculus sceleratus
Ranunculus micranthus
Dicentra canadensis

Summer

Class 1

Lysimachia quadrifolia
Chenopodium album
Impatiens biflora
Stachys tenuifolia
Steironema ciliatum
Teucrium canadense

Class 2

Ammannia coccinea
Amsonia tabernaemontana
Verbena urticaefolia
Eupatorium coelestinum
Polygonum virginianum
Oenothera muricata var. canescens
O. biennis
Galium triflorum
Polygonum pennsylvanicum
Phytolacca decandra

Class 3

Clematis pitcheri	Scutellaria lateriflora
Polygonum longistylum	Anthemis cotula
Solanum carolinense	Mentha piperita
Saururus cernuus	

Fall

Class 1

Eupatorium serotinum	Vernonia missurica
Laportea canadensis	Boltonia asteroides
Vernonia illinoensis	

Class 2

Solidago spp.	Boltonia asteroides var. decurrens
Cuscuta glomerata	
Helenium autumnale	Physostegia virginiana
Boehmeria cylindrica	

Class 3

Solanum nigrum	Aster paniculatus
Matricaria suaveolens	Lobelia cardinalis

FLOODPLAIN PRAIRIE OR SAVANNA.—Unforested areas covered with prairie vegetation were commonly found on the floodplain. A zone of floodplain forest, from one-half to one mile in width, bordered the river channel and surrounded the lakes, whereas the prairie associations occurred on the somewhat higher ground between the river and the bluffs. *Spartina michauxiana* and *Panicum virgatum* constituted the dominant associations of this community.

Prairie areas on a floodplain were included in the investigations by BRENDEL (3) in the Illinois River valley near Peoria, by SAMPSON (11) along the Mississippi River near Savanna, and by CLEMENTS and WEAVER (4) along the western tributaries of the Mississippi River. GLEASON (5) has discussed the relationship of forest and prairie. The writer has discussed the prairie in detail (13).

FLOODPLAIN LAKES.—The lakes of the floodplain are classified as permanent and temporary forest lakes, and prairie lakes. The flood-

plain forest lakes are usually found in the forest near the river channel. The floodplain prairie lakes, lying in the wet prairie, are more frequently farther from the river channel, on higher ground, and shallower than the forest lakes. Each of these types of lakes has a flora which distinguishes it. Here the hydrarch succession from water through slough or swamp to the forest or to the prairie was easily determined.

PERMANENT FOREST LAKE.—Three definite zones of woody plants were observed between the floodplain forest and the permanent forest lake. The first zone retains honey locust and green ash from the floodplain forest. These occur in equal abundance with swamp privet (*Adelia acuminata*) and *Salix nigra*. With these are found also *S. fragilis*, *S. alba*, *S. longifolia*, and *S. amygdaloides*. Rarely does this zone escape submergence to a foot or more for several weeks each spring. The second zone is comprised of *Adelia acuminata* and button bush (*Cephalanthus occidentalis*), together with a few green ash and honey locust trees. Occasionally swamp privet dominates the zone, forming dense, almost impenetrable thickets. This zone is submerged in a foot or more of water from two to six months each year. The third zone consists of an irregular, more or less discontinuous belt of *Cephalanthus*. Except in extremely dry years, this zone is submerged to a depth of a few inches to three or more feet of water during the entire year.

The flora within and surrounding the floodplain lakes of the middle Mississippi River valley (**6**) and the upper Illinois River valley (**3**) is richer in species than is that of the floodplain lakes in the lower Illinois River valley. This may be accounted for by the fact that the condition of the habitat of the lakes in the lower Illinois valley is rendered unstable because of periodic floods which sweep the vegetation from these lakes.

The herbaceous plants occurring within and around the lakes of the lower valley have been separated into three groups: the aquatic plants, which include the floating and submerged plants; the amphibious plants, which comprise floating leaves, submerged, and emerged aquatic plants at the water's edge; and emerged aquatic and swamp plants. The species collected are given in the following list.

HERBS

Aquatic plants

Class 1

Lemna spp.
Wolffia punctata
Spirodela polyrhyza

Class 2

Ceratophyllum demersum
Elodea canadensis
Potamogeton spp.

Class 3

Nelumbo lutea
Pontederia cordata

Amphibious plants

Class 1

Polygonum pennsylvanicum
P. longistylum
Rumex verticillatus
R. crispus

Class 2

Alisma plantago-aquatica
Saururus cernuus
Ludvigia palustris
Radicula nasturtium-aquaticum
Carex spp.

Class 3

Polygonum aquaticum
Sium cicutaefolium
Radicula aquatica
Cicuta maculata
Sagittaria latifolia
S. graminea

Emerged aquatics and swamp plants

Class 1

Radicula palustris
Lippia lanceolata

Class 2

Myosurus minimus
Arabis virginica
Boehmeria cylindrica

Class 3

Ranunculus micranthus	Ranunculus sceleratus
Thlaspi arvense	Iris versicolor
Spermacoce glabra	Equisetum arvense
Bidens bipinnata	Polygonum virginianum
Samolus floribundus	

TEMPORARY FOREST LAKES.—An opportunity is afforded in these shallow temporary forest lakes to observe a more advanced stage in hydrarch succession. What was at one time the outermost part of the lake bed is now occupied by floodplain forest trees. In a zone between these trees and the lake are growing ash, locust, maple, pin oak, and pecan trees. The center of some of these lakes may be occupied by swamp privet, willows, or button bush. These trees and shrubs impede the passage of flood water, and the obstruction causes the deposition of silt. The habitat becomes drier and thereby accelerates the development of the succession to the low-valley climax or floodplain forest.

While three groups of herbaceous plants were found, there were but few species in each group. Three genera of floating aquatic plants were seen, *Lemna*, *Wolffia*, and *Spirodela*. The amphibious plants commonly found were *Saururus*, *Polygonum*, *Jussiaea*, *Radicula*, *Rumex*, and *Sagittaria*. Since these lakes become dry in late summer, the fall aspect of the former water's edge and lake bed is different from that of the permanent forest lake. This space is now occupied by such weed genera as *Chenopodium*, *Bidens*, *Arabis*, *Thalaspi*, and *Rumex*.

FLOODPLAIN PRAIRIE LAKE.—The only woody species found on the wet prairie are an occasional willow tree or clumps of shrubs of willow or button bush. The numbers of herbaceous species of aquatic and amphibious plants of the floodplain prairie lake are about the same, but are more abundant than those of the forest lakes. *Saururus* does not grow here. The following list of herbaceous plants shows that the number of species of submerged and swamp plants of the wet prairie immediately surrounding these lakes is more than twice that of the temporary forest lakes.

HERBS

Emerged aquatic and swamp plants

Class 1

Eleocharis palustris	Chenopodium spp.
Iva ciliata	Eragrostis hypnoides
Xanthium canadense	Panicum virgatum
Juncus effusus	Lippia lanceolata
Rumex crispus	Spartina michauxiana
Alopecurus geniculatus	Bromus secalinus
Amaranthus spp.	Bidens cernua
Carex spp.	Scirpus fluviatilis
Echinochloa crusgalli	Sparganium eurycarpum

Class 2

Scirpus georgianus	Hibiscus militaris
Mimulus alatus	Ambrosia trifida
Alisma plantago-aquatica	Bidens comosa
Sagittaria latifolia	Eclipta alba
Cyperus spp.	

Class 3

Mimulus ringens	Vernonia fasciculata
Cicuta maculata	Ammannia coccinea
Steironema ciliatum	Portulaca oleracea
Eupatorium coelestinum	Penthorum sedoides

Eleocharis palustris is almost always present in this habitat, where it frequently either dominates the muddy shore line or forms dense mats in semi-dry lake beds. *Typha latifolia* occasionally forms rather extensive colonies. *Scirpus fluviatilis* completely fills a few shallow lakes, but it does not seem to have a wide distribution. *Nelumbo lutea* probably once had a wide distribution in the floodplain, judging from the number of seeds and floral receptacles found in dry lake beds. At present it is confined to a few small colonies in drainage sloughs and lake remnants.

Ponds and sloughs have fewer plant species than the larger lakes and are frequently dominated by a single species. They are often

occupied exclusively by *Typha*, *Sagittaria*, *Alisma*, *Polygonum*, *Sparganium*, *Hibiscus*, or *Rumex*.

RIVER BANK.—The plant succession of the river bank or shore line which lies between the water's edge and the floodplain forest may be seen in four zones. The study of the lower zones of this succession is simple, since the fluctuating water level effectively prevents the development of a widely diversified group of life forms.

Within the first zone, which is near the water's edge, seedlings of *Salix*, *Acer*, and *Populus* develop. Most of these seedlings eventually will be destroyed because the water level in most of these habitats is only relatively stable. Herbs growing in this zone are *Polygonum* and *Rumex*, *Eragrostis hypnoides*, *Lippia lanceolata*, *Juncus effusus*, *Eleocharis acicularis*, and *Xanthium canadense*. Less common herbs are *Jussiaea diffusa*, *Alisma plantago-aquatica*, *Sagittaria latifolia*, *Spermacoce glabra*, *Penthorum sedoides*, and *Eclipta alba*. In the second zone are found larger tree seedlings, or sprouts. This zone usually consists mostly of a somewhat dense stand of willows. There occurs in the third zone a series of belts of willow, each belt increasing in age and height as the distance from the water's edge becomes greater. Included in this zone also are a few swamp privets, cottonwoods, and maples. The pioneer trees of the river bank succession are the willow and the cottonwood. The fourth zone, which joins the floodplain forest, is the most stabilized. It is composed of mature willows, cottonwoods, hawthorns, and medium aged maples. *Vitis vulpina*, and to a lesser extent *V. palmata* and *V. cordifolia*, often form dense mats over the willow fringe. The trees that grow along the shore in the order in which they most commonly occur are *Salix nigra*, *S. fragilis*, *S. amygdaloides*, *S. discolor*, *Populus deltoides*, *Acer saccharinum*, *Adelia acuminata*, *Salix alba*, *Crataegus* sp., *Salix longifolia*, and *Platanus occidentalis*.

The following herbs were observed on the more stabilized zones of the river bank:

HERBS

Spring

Class 1

Radicula palustris	Myosurus minimus
Senecio glabellus	Equisetum arvense

Class 2

Arabis virginica	Thlaspi arvense
Sisymbrium canescens	Lepidium virginicum

Class 3

Ranunculus abortivus	Chaerophyllum procumbens
Potentilla monspeliensis	

Summer

Class 1

Polygonum pennsylvanicum	Polygonum lapathifolium
	Rumex crispus
Chenopodium album	Chenopodium glaucum
Lippia lanceolata	Convolvulus sepium

Class 2

Spermacoce glabra	Polygonum longistylum
Oenothera muricata	P. aviculare
Gonolobus laevis	Mimulus alatus

Class 3

Alisma plantago-aquatica	Baptisia leucantha
Jussiaea diffusa	Hibiscus militaris
Samolus floribundus	

Fall

Class 1

Amaranthus retroflexus	Eupatorium serotinum
A. blitoides	Eragrostis hypnoides
Carex spp.	Bidens comosa
Eleocharis acicularis	Juncus effusus
Xanthium canadense	

Class 2

Acnida tuberculata	Bidens frondosa
Amaranthus graecizans	Commelina communis
Portulaca oleracea	Echinochloa crusgalli
Polygonum amphibium	Sagittaria latifolia
Bidens bipinnata	Eclipta alba
Penthorum sedoides	

Class 3

Bromus secalinus	Gratiola virginiana
Cuscuta compacta	Mimulus ringens
Bidens cernua	Eleusine indica
Polygonum hydropiperoides	Radicula armoracia

Both primary and secondary successions are represented along the river bank. Primary succession is observed below the mouth of entering tributaries where the bank is encroaching upon the river. Secondary succession is constantly taking place in the zones nearest the water's edge because of the almost annual destruction of the river bank plants. Ice jams along the river contribute largely to this destruction.

ISLANDS.—The flora of the islands is separated into two groups, that of the small and that of the large islands. The small islands have a maximum length of 800 yards, while the large islands vary from 800 yards to 3 or 4 miles in length, and may have a maximum width of 1 mile.

The small islands are characterized by a pioneer type of vegetation, and show the earliest stages in the hydrarch succession; the larger islands include a more advanced stage in this succession. The islands are generally from 1 to 3 feet lower than the floodplain of the mainland. This fact may explain why the succession on the largest islands does not reach the final stage of the climax floodplain forest.

The farming that was attempted on several of these islands met with the same or even less success than that on the unleveed floodplain. Abandoned farm areas showed present stages of plant growth in which the plant species corresponded with those of the primary plant succession of the islands.

SMALL ISLANDS.—The small islands are irregularly oval in shape, rounded at the upper end and tapering toward the downstream end. Since the upper end of an island is the oldest and wears away while the lower end builds up, zonation and well defined steps of plant succession were easily recognized.

Total tree counts were made in ten small islands and the results are recorded in figure 1. The lower tip of these islands has the

pioneer stage or first zone (*A*) of the plant succession. This first zone, which is identical with those found at the water's edge on the mainland, forms the outermost plant fringe of the entire island.

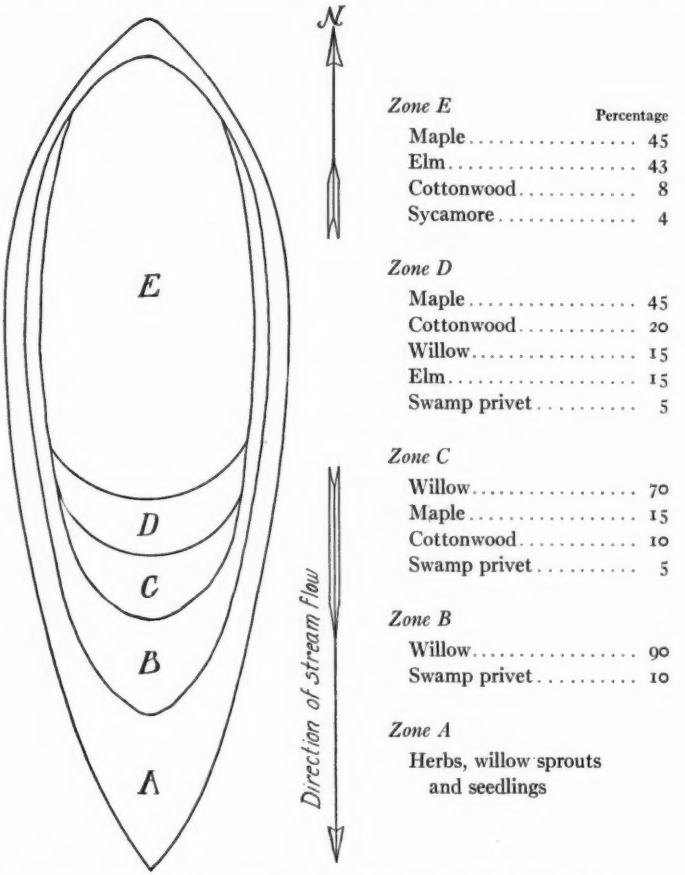

FIG. 1.—Floral analysis of small islands in lower Illinois River valley

Farther up from the lower end of the island occurs the second zone (*B*), which is a consociation of young willows with a few swamp privets and cottonwood sprouts. In the third zone (*C*) the per-

centage of willows decreases, cottonwoods become more important, and young maples appear. The fourth zone (*D*) exhibits two features, the occurrence of elms and the peak of cottonwood concentration. Willows decrease abruptly in this zone. Maples are found in approximately the same proportion on the upper part of the islands. In the fifth zone (*E*) the upper and interior three-fifths of the island has attained a condition of relative stability. Soft maples and elms are dominant, with the former slightly more numerous; cottonwoods decrease to 6–10 per cent of the stand; sycamores occur occasionally. The herbaceous cover differs little from that of the lower floodplain forest of the mainland.

LARGE ISLANDS.—At their lower end, the large islands exhibit the same general type of zonation as the small islands. The forest on the upper end differs primarily from that of the small islands in having an increased number of species and in having attained a state of relative stability. This most stable zone may be designated as the sixth zone or climax island forest. Although essentially the same species occur here as in the climax floodplain forest, the data in table VI show that the dominance index of some of the species in the two habitats varies.

The first five tree species listed in table VI are the most abundant in both the sixth zone or island climax forest and the climax floodplain forest of the mainland. The dominance index for these island species is somewhat less for the island than for the mainland species, except for the elm, which becomes the dominant tree. On the other hand, among the subdominant species the cottonwood, honey locust, and the sycamore attain a much higher dominance index in this habitat than on the mainland. Apparently the elm and the three species just given are more water tolerant and have a wider adaptation to soils containing less humus than have the other species. The hackberry, ash, hawthorn, river birch, swamp privet, and persimmon are subdominants which are more widespread and occur in greater numbers in the climax floodplain forest.

The herbaceous plants of the large islands are the same species as those found in the upper part of the small islands and in the low floodplain forest of the mainland.

LEVEES.—Plant succession on artificial levees in the valley has been discussed elsewhere (12).

TABLE VI

QUANTITATIVE EVALUATION OF TREES OF GREATEST FREQUENCE ON LARGER ISLANDS, AND COMPARISON OF DOMINANCE INDEX WITH FLOODPLAIN FOREST

SPECIES	NO. QUADRATS CONTAINING SPECIES	AVERAGE NO. INDIVIDUALS PER QUADRAT	DOMINANCE INDEX	
			ISLAND FOREST	FLOODPLAIN FOREST
Ulmus americana	50	7.3	365	320
Acer saccharinum	50	7.2	360	418
Carya illinoensis	46	3.7	170	193
Adelia acuminata	37	3.0	111	133
Quercus palustris	25	4.0	100	153
Populus deltoides	34	2.9	90	24
Celtis occidentalis	41	2.1	86	114
Gleditsia triacanthos	26	2.0	52	32
Platanus occidentalis	31	1.5	47	Rare
Fraxinus pennsylvanica var. lanceolata	12	2.0	24	109
Crataegus sp.	23	1.0	23	115
Betula nigra	13	1.0	13	69
Diospyros virginiana	12	1.0	12	26

TREES OF INFREQUENT OCCURRENCE

Carya laciniosa
Acer negundo
Morus rubra

Summary

1. Soil types found in Pike and Calhoun counties at the lower end of the Illinois River were correlated as far as possible with the plant communities growing upon them. The soils in the entire area studied have rather a wide variety of texture and type. There was a small range in the hydrogen-ion concentration of the soils. The variation of the pH of the upland soils was from 6 to 6.6; the floodplain soils was from 7 to 7.8. The pH of the talus slope was 8. The narrow range of pH seems to indicate that it is not an important factor in limiting the distribution of species.

2. A transect of the lower valley affords four general divisions: upland, bluff, transition region, and floodplain. Subdivision of these

results in ten major plant communities: upland pasture and old fields, upland forest, limestone bluff, hillside-talus slope forest, talus slope-floodplain transition forest, floodplain forest, floodplain prairie, floodplain lakes and sloughs, river bank, and islands.

3. The dominance index, derived from a quantitative study of trees 6 inches in diameter breast high and of shrubs over 6 feet in height, was used to determine the types of forest in this region. Herbs and some of the woody plants under 6 feet in height were studied, in the various habitats, in their spring, summer, and fall aspects.

4. The fragments of the original upland prairie that persist between the present areas of pastures and old fields contain few of the prairie plants that were formerly abundant. However, this upland habitat is second richest of all within the range in number of species when introduced ruderals are recorded.

5. The upland forest is a typical midwestern oak-hickory forest. Of all the plant communities, this upland forest ranks fourth in total number of species.

6. The limestone bluff has the smallest number of species of any of the plant communities studied and nearly all of these are herbs which appear in the spring.

7. The hillside-talus slope forest has as its dominant species the hard maple, the chestnut oak and the red oak. This plant community is the richest of all in both herbaceous and woody species. The explanation for this lies perhaps partly in the fact that it has been least disturbed by man, and partly because it is a favorable habitat for a great number of mesophytic plants indigenous to the central states.

8. The talus slope-floodplain transition forest lies above the zone of destructive floods. Cultivation and grazing have brought about the destruction of many of the native species. It contains species from both the hillsides and the floodplain. The elm, the sycamore, and the hackberry are the dominant trees.

9. The floodplain forest has more trees that have a dominance index of 100 or more than has any other forest studied. Soft maple and elm are the dominant trees. The small number of woody plants in the floodplain forest compared with other forests may be accounted

for by the scarcity of shrubs. Woody plants comprise 26 per cent of the total plants recorded for the floodplain forest, while hillside-talus slope forest and talus slope-floodplain forest have 33 per cent. The destructive action of floods doubtless accounts for the small number of perennial herbs and shrubs in this forest.

10. The floodplain prairie or savanna occurs on the broader floodplain areas of the Illinois River, generally a few feet higher than the floodplain forest; hence it is drier and less subject to flooding.

11. The lakes and sloughs have been greatly reduced in number and size by drainage. Both the permanent and temporary floodplain forest lakes and the floodplain prairie lakes show the usual zonation of plants in the hydrarch succession from the water to the forest or to the prairie climax. In general, the number of plant species is much smaller than in many lakes elsewhere, possibly because of the annual destruction of the plants by floods.

12. Four zones of plants constituted the hydrarch succession which occupies the river bank between the water's edge and the floodplain forest. Willow and cottonwood were the pioneer trees. In the third zone the willow occurred in several belts, each of which increased in age and height as the distance from the water's edge became greater.

13. The small islands have a zonation which is similar to that of the river bank. Except at the center of the islands, the habitat is relatively unstable. A quantitative study of the trees in each zone showed that there was a gradual change in tree species, and in the frequency of their occurrence from the less stable zone at the water's edge to the more stable zone at the center of the islands.

14. The lower end of the large islands has a zonation similar to that of the small islands. At the upper end the habitat is more stable, and there is a greater number of plant species than in this position on the smaller islands. A comparison of the dominance index of the trees in the climax island forest and the climax floodplain forest of the mainland indicates that the former has not reached so high a state of development as the climax forest of the mainland.

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HYDROTROPIC RESPONSES OF ROOTS IN SOIL

W. E. LOOMIS AND L. M. EWAN

(WITH FOUR FIGURES)

A positive response of roots to moisture stimuli is very commonly assumed. The literature shows that this assumption is based largely upon the observations of SACHS (5) and MOLISCH (4), who showed that roots growing through the screen bottom of a moss-filled basket turned at an angle of 45° from the vertical and followed the moist surface of the bottom of the basket; and upon the researches of HOOKER (3), who obtained positive responses of roots to moisture when they were grown in a water vapor gradient and out of direct contact with a moist surface.

SACHS attempted to explain this bending by assuming that the exposed surface of the roots lost more water by evaporation and was therefore cooled below the inner surface, which was in contact with the moist surface. He attempted, with negative results, to obtain a bending away from the moist surface by warming the roots with direct radiation. MOLISCH assumed also that the evaporation on the exposed side of the roots was responsible for the stimulus but he did not attempt to introduce temperature factors. All who have attempted the SACHS experiment know that the "hydrotropic response" of the roots under these conditions depends upon a moderate moisture deficit in the air. As the humidity of the air surrounding the roots approaches 100 they grow downward and show no tendency to follow the moist screen.

In some preliminary experiments, the writers found that when roots were grown in a saturated atmosphere and showed no tendency to follow a moist surface, the temperature of the moist surface was the same as that of the air. When the humidity was decreased to 70 or 80 per cent, the roots of corn followed a moist filter paper at an angle of 45° and the temperature of the filter paper was cooled by evaporation to 5° below air temperature. These results suggest that

the bending of roots in air is associated with temperature differences. The side of the root in contact with a cool moist surface may grow more slowly because of its lower temperature and the unequal growth rate on the two sides of the root cause it to bend toward the moist surface against the effect of geotropism. In HOOKER's (3) experiments the roots were hung vertically in front of a moist blotting paper at distances as great as 10 cm. from the paper. Positive responses were shown at moderate moisture vapor gradients and negative responses at higher gradients. The positive responses were called hydrotropism and the negative responses were assigned to greater drying and less turgor on the side of the root away from the moist surface, an effect which became dominant as the moisture gradient was increased. Temperature differences were not measured but were undoubtedly present.

The hydrotropic responses of roots in air are of considerable academic interest, but they appear to have little relationship to the development of plants under normal conditions. The minimum soil moisture to which plant roots are normally exposed is represented by the wilting percentage or the wilting coefficient of the soil. The wilting percentage is considerably (47 per cent) above the hygroscopic percentage, at which point the soil moisture is stated to be in equilibrium with air saturated with water vapor. It would not be expected, therefore: (1) that vapor gradients would normally be present in soil regions where plant roots were growing; (2) that evaporation rates on the two sides of a buried root would differ appreciably; or (3) that temperature factors could be expected to enter into the normal hydrotropic responses of roots in soils. So far as the writers have been able to determine, no experiments bearing directly on the problem of the hydrotropic responses of roots in soil have been published, and the explanations which have been assigned to hydrotropic responses in air not being applicable to soil conditions, the question arises as to whether hydrotropism is a normal response of roots growing in soil.

Methods

The findings of HENDRICKSON and VEIHMEYER (2), that a soil at the moisture equivalent, sometimes called the field coefficient, does

not lose moisture by capillary action to dry soil in intimate contact, suggested the method used in these studies. A large sample of sandy loam soil with an observed wilting coefficient of 7.1 per cent was air dried to 4 per cent moisture, and one-half of the soil was then moistened to 11.8 per cent. This moisture percentage appeared to be nearly optimum for plant growth as determined by the crumbling test; seeds germinated in samples of the soil grew readily until its moisture content was reduced to the wilting point. When a sample of the dry soil was packed into a cylinder, moistened from the top, and allowed to stand for three weeks, a clear-cut and practically stationary line between moist and wet soil was observable. The soil was removed by layers and its moisture content determined. The upper 10 cm. averaged 13.86 per cent moisture and was uniform within the limits of error in determination. Between 10 and 16 cm. the moisture content decreased from 13.86 to 11.9 per cent, and between 16 cm. and the observable moist-dry line at 19 cm. the moisture content decreased again from 11.9 to 9.2 per cent. Below the division line the moisture content was 5.2 per cent and dropped off in 4 cm. to the 4.0 per cent of the experimental soil. Under the conditions in the tube, 13.86 per cent might be taken as the field coefficient, although after three weeks there was some movement from the lower layers of the moist soil either by greatly reduced capillary action or by vaporization, until the moisture content of the soil above the dividing line reached a minimum of 9.2 per cent. These maximum and minimum values of 13.86 and 9.2 per cent may be compared with a calculated moisture equivalent of 13.0 per cent, using the equation of BRIGGS and SHANTZ (1). The moisture content of the adjoining dry soil was also built up, obviously by vapor movement, to a value slightly above the calculated hygroscopic coefficient of 4.83 per cent. With the moisture percentage of 11.8 per cent which was used in these experiments, and with most of the experiments completed in one rather than in three weeks, clear-cut wet and dry lines such as are shown in figures 2, 3, and 4, which had not moved appreciably from the original point, were obtained in all experiments. We were thus able to obtain a sharp moisture content line, with probably some gradation over a narrow zone as the result of vapor movement from

the moist into the dry soil layer. The roots of various plants were then grown so that the effect of this steep soil moisture gradient on the direction of growth could be observed.

Experimental results

Roots grown between wet and dry soil

In early experiments the central portion of a shallow box was filled with the moistened soil and the ends with the dry soil, except for grooves which were dug on either side, lined with 14-mesh wire cloth, and packed with the moist soil. The boxes were placed in a moist chamber and germinating seeds of peas and corn planted in the ends of the grooves. The roots of the developing seedlings were thus surrounded on three sides and the bottom by soil below the wilting coefficient, and on the fourth side by moist soil which connected to the larger area of moist soil in the center of the box. The development of plants under these conditions is illustrated in figures 1 and 2. The radicles grew straight downward through the screen wire into the dry soil and were arrested in their development. Branch roots which grew into the dry soil were checked without any evidence of bending. Branch roots which developed in the direction of the moist soil continued until by chance they grew into the dry soil. The check in the growth of the roots growing into the soil of low moisture content tended to result in profuse branching, and those branches which in their normal arrangement were pointed toward the moist soil continued the spread of the root system. Such a trial and error method resulted in some of the roots reaching the center of the flat, and with continued secondary development it might have appeared in a short time as though such roots had developed by determination rather than by accident.

Roots grown between vertical masses of wet and dry soil

In a second series of experiments a shallow flat was filled with the moist soil, the seeds to be observed were placed on the surface, and the frame of a second flat was set on top of the first, filled with the dry soil, and a cover clamped in place. The soil was surrounded by heavy paraffined paper to prevent changes in moisture content and the boxes were placed in moist chambers for 6–10 days to allow the

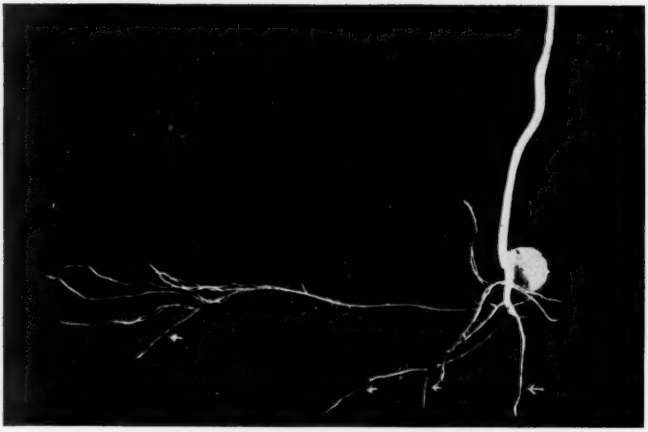

FIG. 1

FIG. 2

FIGS. 1, 2.—Fig. 1, seedling of *Pisum sativum* grown with moist soil on the left and with dry soil on the other sides and below the plant. At points indicated by the arrows the roots grew into the surrounding dry soil and were checked in their development. No hydrotropic response is shown. Fig. 2, plants of same growing from wet to dry soil without indication of hydrotropism in either primary or secondary roots. Note that while some roots bend toward the moist soil, others bend toward the dry.

roots to develop. Some of the boxes were placed vertically so that the roots would start downward between layers of moist and dry soils. Others were placed at an angle of 45° with the dry soil on the lower side of the box. Under these conditions the geotropic response of the roots would tend to cause growth into the dry soil and a hydrotropic response sufficient to overcome the effect of gravity would be shown if the roots followed the 45° angle of the moist-dry soil division line. This last experiment was intended to simulate the experiments of SACHS, but under soil conditions. Since the wilting coefficient of the soil used was 7.1 per cent and the hygroscopic coefficient approximately 5.0 per cent, the development of the roots which grew into the soil containing 4.0 per cent moisture was quickly checked. Roots which grew into the moist soil continued normally for the duration of the experiment. This situation is well illustrated in figure 3, where the secondary roots of the bean plant on the lower or dry side are short while those in the moist soil are 10 to 20 cm. long. The relative humidity of the soil air at the different moisture levels was not measured, but since the moisture percentage of the dry soil was below the hygroscopic coefficient, it may be assumed that the contained air was unsaturated with water vapor. In contrast to this situation, the air of soil at the wilting percentage would be saturated with water vapor, so that our experiments should have afforded abnormally favorable conditions for either hydrotropic or hygrotropic responses.

Some 3000 seedlings representing 26 species were planted between vertical masses of moist and dry soil, and the bending of the primary roots was observed after approximately one week of growth. Readings were made by removing very carefully the layer of dry soil and counting first the roots whose tips had bent into the dry soil; second, those which were growing vertically on the moist-dry line; and third, those roots which had turned into the moist soil. Since the roots which turned toward the soil of lower moisture content were quickly checked in their development, while those which turned into the moist soil grew several centimeters, it was difficult to obtain comparable readings for these two classes. Few, if any, of the roots which turned into the moist soil were missed in the counts, while many of those which turned in the opposite direction were so short that they

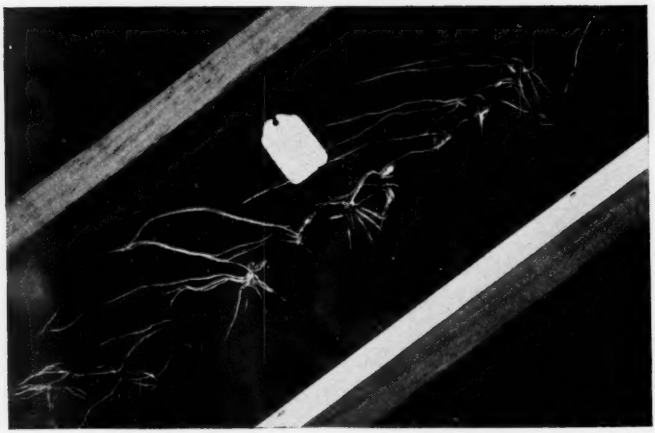

FIG. 3

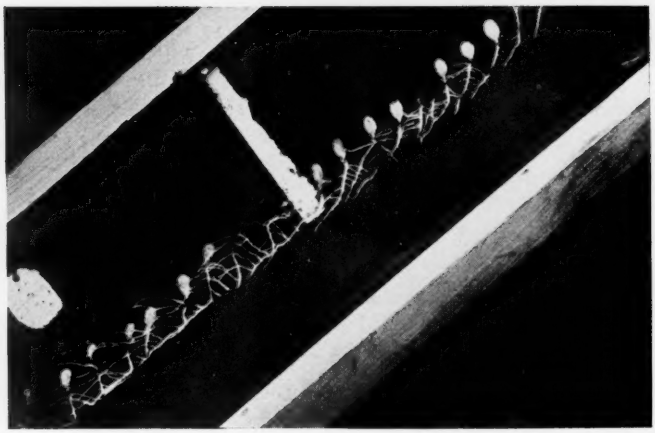

FIG. 4

FIGS. 3, 4.—Fig. 3, roots of *Phaseolus vulgaris* showing the much greater growth of secondary roots which grew by chance into the moist soil and the failure of those which turned toward the dry soil to show any hydrotropic response. Fig. 4, *Cucurbita pepo*, lower one-half, and *Citrullus vulgaris* seedlings, upper one-half, showing positive and weakly positive hydrotropic responses. The strongly positive response shown by the *C. pepo* seedlings appears to be the exception rather than the normal reaction of plant roots in soil. Note that secondary roots show no hydrotropic response.

were disturbed in removing the soil and could not be accurately counted. This difficulty probably explains a noticeable tendency in all of the experiments for a slightly higher percentage of roots in the wet soil. The complete data are given in table I. Of the Gramineae used, only one species, a sample obtained as Egyptian millet and assumed to be a variety of *Setaria italica*, was definitely positive in its reaction as shown by a tendency of the roots to bend from the vertical into the moist soil. Of the legumes, two species of lima beans and one variety of *Phaseolus vulgaris* appear to have been positive, as was also *Fagopyrum esculentum* in the miscellaneous group. The data for 25 of the 30 species and varieties are considered to indicate no significant tendency for the roots to bend toward the moist soil. These 25 varieties include 11 families and 19 genera of seed plants.

A failure of the root to respond to a moisture gradient of the type used in this experiment might be considered strong evidence against the importance of hydrotropism in soils. Certainly the bending required to count as a positive response would be very little affected by the forces of gravity. On the other hand it is possible that the moisture conditions, as a result of vapor movement, were sufficiently favorable on both sides of the growing roots so that little or no hydrotropic response was evidenced. This last point was checked by noting the ability of roots to follow a wet-dry soil line at an angle of 45°.

Roots grown between wet and dry soil at an angle of 45°

The seeds for these experiments were planted in the same manner as were those for the last series, so that they were in contact on one side with dry soil and on the other with moist soil. Germination and development were not always good under these conditions and it is possible that many roots which broke through the seed coat directly into the dry soil did not make sufficient growth to give a fair test of their hydrotropic response. Nevertheless the varieties which showed some evidence of hydrotropism in the first experiments showed also some response in these tests.

The normal tendency of the roots was to grow directly downward into the dry soil. This type of response is shown in figure 2 where

TABLE I

HYDROTROPIC RESPONSE OF ROOTS GROWN BETWEEN VERTICAL MASSES OF WET AND DRY SOIL

PLANT	TOTAL NO. PLANTS	HYDROTROPIC RESPONSE IN PERCENTAGE OF ROOTS		
		POSITIVE	NONE	NEGATIVE
Zea mays-indentata	177	27.0	53.2	19.8
Triticum aestivum	84	19.1	60.7	20.2
Hordeum vulgare	93	7.5	82.8	9.7
Zea mays-rugosa	108	41.7	14.8	43.5
Holcus sorghum-technicus	165	8.5	89.1	2.4
Gramineae	627	20.8	60.1	19.1
Pisum sativum	182	8.5	77.5	14.0
Glycine max	129	19.4	64.3	16.3
Phaseolus coccineus	84	7.1	85.8	7.1
Phaseolus vulgaris (Michigan pea)	43	18.6	62.8	18.6
Phaseolus vulgaris (stringless green pod)	182	21.5	68.1	10.4
Lupinus hartwegii	152	5.9	88.2	5.9
Vigna sinensis	123	13.8	73.9	12.3
Vicia sativa	53	5.7	90.5	3.8
Leguminosae	948	12.6	76.4	11.0
Beta vulgaris (mangles)	42	2.4	97.6	0.0
Beta vulgaris (sugar beet)	95	22.1	54.7	23.2
Spinacia oleracea	157	2.5	94.3	3.2
Helianthus annuus	142	7.0	82.4	10.6
Tragopogon porrifolius	61	1.6	98.4	0.0
Linum usitatissimum	150	6.7	79.3	14.0
Ipomoea sp.	128	18.0	62.5	19.5
Raphanus sativus	249	6.3	89.5	4.2
Citrullus vulgaris	116	20.7	58.6	20.7
Tetragonia expansa	9	11.1	77.8	11.1
Hibiscus esculentus	44	20.4	61.4	18.2
Pinus sp.	114	0.0	100.0	0.0
Miscellaneous	1307	9.9	79.7	10.4
Fagopyrum esculentum	295	30.8	57.7	11.5
Phaseolus lunatus	54	42.6	27.8	29.6
Phaseolus limensis	59	42.4	52.5	5.1
Phaseolus vulgaris (Red kidney)	38	47.4	42.1	10.5
Setaria italica	44	61.4	36.3	2.3
Miscellaneous	490	44.9	43.3	11.8

pea roots have grown into the dry soil, and bending toward the dry side is as common as bending toward the moist. Three other types of development are possible: (1) the roots might grow horizontally into the moist soil; (2) they might grow downward at an angle of 45°, following the moist-dry line; or (3) they might assume some intermediate position between the vertical and the 45° bending. The first classification of roots growing horizontally was not observed except in some of the grasses where horizontal growth is reasonably common. In these, as will be explained later, the direction of growth of the root depended primarily upon the orientation of the seed. The bending of the roots at the moist-dry line and their growth downward at an angle of 45°, as illustrated in the lower half of figure 4, are designated in the tables as a strong positive response. This response is comparable with that obtained in the SACHS experiment. The upper portion of figure 4 illustrates the intermediate response in which the roots of watermelon bent toward the moist soil but did not follow the moist-dry line. This response is designated in the tables as a weak positive response. Typically the roots in this group bent at an angle of $22\frac{1}{2}°$ from the vertical. The complete data for these experiments are given in table II.

Two species of *Phaseolus* may be considered to have shown a positive response when 16.4 and 13.2 per cent, respectively, of their roots followed the moist line. Four other species showed a weaker positive response in that from 10.5 to 32.8 per cent of their roots bent toward the moist line but did not follow it. The results with the grasses are disregarded because the normal tendency of many of the primary roots of the grass seeds, and particularly of the primary roots of maize, is to grow downward at an angle of 45°. When corn was placed with the germ toward the moist soil and the tip downward, it was found that 90 per cent or more of the roots stayed above the 45° line and grew into the moist soil. When the germ was placed toward the dry soil, the same proportion grew below the 45° line into the dry soil. When the tip of the kernel was toward the top of the box between vertical masses of wet and dry soil, 49 per cent of the radicles bent into the dry soil and 51 per cent into the wet. If we consider the seven species of legumes and the nine miscellaneous species in the experiments reported in table II, we find that: (1) there was no tend-

ency for the roots to grow into the moist soil above the 45° line; (2) with two or possibly three species there was a significant tendency to follow the 45° line; (3) not more than one-third of the species showed any evidence of a hydrotropic response under the conditions of this experiment.

TABLE II

HYDROTROPIC RESPONSE OF ROOTS GROWN BETWEEN WET AND DRY SOIL AT AN ANGLE OF 45°

PLANT	TOTAL NO. PLANTS	RESPONSE IN PERCENTAGE ROOTS			
		NONE	WEAK POSITIVE	POSITIVE	INTO WET SOIL
Zea mays-indentata	106	63.2	.....	17.5	19.3
Zea mays-rugosa	123	69.1	.....	18.7	12.2
Triticum aestivum	65	69.2	.....	27.7	3.1
Holcus sorghum-technicus	148	100.0	0.0	0.0	0.0
Gramineae	442	75.4	.....	16.0	8.6
Pisum sativum	225	100.0	.....	0.0	0.0
Glycine max	188	98.9	.....	1.1	0.0
Phaseolus lunatus	167	67.2	16.4	16.4	0.0
Phaseolus limensis	58	58.6	32.8	8.6	0.0
Phaseolus vulgaris	28	82.1	14.3	3.6	0.0
Phaseolus vulgaris	144	86.8	.....	13.2	0.0
Phaseolus coccineus	76	89.5	10.5	0.0	0.0
Lupinus hartwegii	252	100.0	0.0	0.0	0.0
Leguminosae	1138	85.4	9.2	5.4	0.0
Helianthus annuus	239	100.0	0.0	0.0	0.0
Beta vulgaris	107	97.2	.....	2.8	0.0
Spinacia oleracea	252	100.0	0.0	0.0	0.0
Linum usitatissimum	197	99.5	0.0	0.0	0.5
Ipomoea sp.	61	100.0	0.0	0.0	0.0
Raphanus sativus	239	100.0	0.0	0.0	0.0
Citrullus vulgaris	113	100.0	0.0	0.0	0.0
Tetragonia expansa	13	100.0	0.0	0.0	0.0
Hibiscus esculentus	71	88.7	11.3	0.0	0.0
Fagopyrum esculentum	133	89.4	8.3	2.3	0.0
Miscellaneous	1425	97.5	1.95	0.5	0.05

ROOTS GROWING FROM MOIST INTO DRY SOIL WITH A 45° SLOPE IN MOIST-DRY LINE

In this series of experiments the seeds were covered with 1–2 cm. of moist soil (figs. 2–4) so that normal germination and initial growth

of the radicle were obtained. After the radicles had grown vertically for 2–3 cm. they reached the line of the dry soil and their response was measured as "none" if they grew vertically into the dry soil until the growth was checked (fig. 2); as "weakly positive" if they bent perceptibly toward the moist soil but did not follow the 45° line (upper portion of fig. 4); and as "positive" if they followed the moist line (lower portion of fig. 4). Under these more favorable growing conditions, results as shown in table III were more strongly positive although no tendency was observed for the roots to grow up into the moist soil above the wet-dry line. One variety of *Phaseolus vulgaris* gave what may be considered a normal positive, hydrotropic response. Another variety of *P. vulgaris*, one of *P. limensis*, and one experiment with *Vigna sinensis* showed a positive reaction in 20 per cent or more of the roots. Among non-legumes a miscellaneous group representing six families is considered to be without measurable hydrotropic response. A group representing five families showed weak response, and a group representing three families a strong response with an average of two-thirds of the roots following the wet-dry line. In several instances, duplicate experiments have been shown separately in the data rather than averaged. It will be observed that in general the agreement is good. Two experiments with *Cucumis sativus* check closely, two with *Tragopogon porrifolius* even more closely, two with *Vigna sinensis* moderately well, but three with *Fagopyrum esculentum* showed a considerable variation so that one experiment is thrown into the group with moderate response and two into the group with positive response.

The average response in these experiments is considerably higher than in the first or second series. We consider that conditions here were more nearly normal for the development of the seedlings and more favorable for hydrotropic responses. In spite of this there appeared to be decided variations in the reaction of various species and indications of variations between varieties of the same species. Several experiments were run to check upon the possibility that, in spite of our care to maintain uniform conditions, the observed variations may have been due to chance differences in experimental technique. Three species which had shown strong positive responses in the earlier experiments and three which had shown negative responses

TABLE III

RESPONSE OF ROOTS GROWING FROM MOIST INTO DRY SOIL WITH 45° ANGLE*

PLANT	NUMBER OF SEEDLINGS	RESPONSE IN PERCENTAGES OF PLANTS		
		NO RESPONSE	WEAKLY POSITIVE	STRONGLY POSITIVE
Phaseolus lunatus	17	88.2	11.8	0.0
Pisum sativum	65	95.4	4.6	0.0
Pisum sativum	49	96.0	2.0	2.0
Robinia pseudo-acacia	31	87.1	9.7	3.2
Lens esculenta	57	84.2	7.0	8.8
Glycine max	64	93.7	4.7	1.6
Leguminosae	283	90.8	6.6	2.6
Vigna sinensis	55	76.4	20.0	3.6
Vigna sinensis	34	55.9	23.5	20.6
Phaseolus limensis	64	42.2	32.8	25.0
Vicia sativa	25	68.0	16.0	16.0
Phaseolus vulgaris (Mich. pea)	46	43.5	23.9	32.6
Leguminosae	224	57.2	23.2	19.6
Phaseolus vulgaris (green pod)	59	11.9	5.1	83.0
Beta vulgaris	32	93.8	0.0	6.2
Anethum graveolens	64	82.8	10.9	6.3
Allium cepa	17	88.3	11.7	0.0
Tragopogon porrifolius	64	98.5	1.5	0.0
Tragopogon porrifolius	56	98.2	0.0	1.8
Cucumis sativus	44	95.5	0.0	4.5
Cucumis sativus	19	94.7	5.3	0.0
Pinus sp	31	96.8	3.2	0.0
Miscellaneous	327	93.6	4.1	2.3
Fagopyrum esculentum	40	60.0	12.5	27.5
Raphanus sativus	68	64.7	26.5	8.8
Beta vulgaris	61	39.3	32.8	27.9
Hibiscus esculentus	32	62.5	21.9	15.6
Citrullus vulgaris	59	54.3	22.0	23.7
Miscellaneous	260	56.2	23.1	20.7
Setaria italica	54	22.2	24.1	53.7
Fagopyrum esculentum	62	30.7	14.5	54.8
Citrullus vulgaris	37	8.1	16.2	75.7
Fagopyrum esculentum	55	10.9	5.5	83.6
Cucurbita pepo	25	8.0	16.0	76.0
Miscellaneous	233	16.0	15.3	68.7

* Seedlings grew through 1.5 cm. of moist soil into dry soil lying with 45° slope. "No response" grew straight into dry soil; "weak positive" response turned toward moist soil at angle of about 22° but grew into dry; "positive" response followed line of moist soil at 45° angle.

were planted in pairs in three flats. Each flat was planted in alternating rows, one-half to a positively and one-half to a negatively responding species. The data in table IV show that there was unquestionably a pronounced variability in the reaction of these species. The differences when the two plants were grown in the same flat were comparable with the differences obtained in the other experiments where ordinarily two varieties were planted in each flat, but they were chosen on the basis of convenience in seeding and reading the results rather than on the basis of the reaction of the plants.

TABLE IV

VARIATIONS IN HYDROTROPIC RESPONSE
FLATS PLANTED WITH ALTERNATING ROWS OF TWO SPECIES AND HELD AT ANGLE OF 45°

PLANT	FLAT NO.	PERCENTAGE OF TOTAL SHOWING		
		NO RESPONSE	WEAKLY POSITIVE	STRONGLY POSITIVE
Tragopogon porrifolius	1	98.5	1.5	0.0
Citrullus vulgaris	1	8.1	16.2	75.7
Cucumis sativus	2	95.5	0.0	4.5
Fagopyrum esculentum	2	10.9	5.5	83.6
Cucumis sativus	3	94.7	5.3	0.0
Cucurbita pepo	3	8.0	16.0	76.0

Summary

1. Seven thousand seven hundred sixty-three seedlings representing 29 genera and 14 families have been grown in a great number of experiments to determine the hydrotropic response of primary roots of seedlings growing on a steep soil moisture gradient. The steep gradient has been obtained by placing in contact a layer of soil with a moisture percentage below the hygroscopic coefficient and a layer with a moisture percentage slightly below the moisture equivalent or field coefficient. This gradient was intended to provide optimum conditions for a hydrotropic response, and is steeper than will ordinarily be encountered in the field where the wilting percentage normally represents the low moisture content of the areas into which roots might grow. Under these conditions the growth of seedlings in

the moist soil has been normal and a sharp moist-dry line between the two soil layers has been maintained over a period of one to two weeks. In contrast to the normal growth of roots in the moist layer, growth has been completely checked in roots which have penetrated more than approximately 1 cm. into the dry soil.

2. The primary roots of many plant species have shown no tendency to bend when growing from the moist soil layer with available moisture into the dry soil with insufficient moisture to maintain growth. The typical response in this group of plants has been a growth along the lines determined by the position of the seed and the normal response to gravity, until the root has grown into the dry soil. Under these conditions branching was stimulated in the portions of the root remaining in the moist soil. These branches grew normally outward and down without showing hydrotropic response, and continued in the moist soil or passed from it into the dry soil, depending upon their initial direction of growth. Those which grew into the dry soil, with favorable food conditions, again formed branches which repeated the response of the earlier roots.

3. A second group of plants showed weak hydrotropic responses. A small percentage of the primary roots bent at an angle of 45° from the vertical and followed a moist-dry soil line. Normally a somewhat larger percentage showed some bending toward the more moist soil but did not follow the line at an angle of 45°.

4. A third and smaller group of plants showed responses in soil comparable with those obtained in air with the SACHS experiment, with from one-half to three-fourths of the roots following the moist soil at an angle of 45° and another fraction bending toward the moisture but not following the 45° line. Secondary roots as a group showed no clear-cut hydrotropic response in any of these experiments.

5. The data indicate that hydrotropism in roots is by no means comparable in distribution and intensity of reaction with geotropism in roots and stems or with phototropism in stems. The results with several species of the Cucurbitaceae and Leguminosae suggest that hydrotropic responses may depend upon a genetic factor present in some plants and absent from closely related species or varieties.

6. Apparently these results demonstrate the possibility of a re-

sponse in soil similar to that which has been obtained in the air. On the other hand, they seem to show equally clearly that hydrotropism is not a universal and probably under field conditions not a common plant response.

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EFFECTS OF NITROGEN SUPPLY ON RATES OF PHOTOSYNTHESIS AND RESPIRATION IN PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 471

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Introduction

There is a conspicuous lack of recorded results of experiments to determine the effect of variations in the nutrient supply of plants on their photosynthetic and respiratory rates. SPOEHR and MCGEE (7), working with excised leaves of *Helianthus*, found that if the petioles were immersed in nutrient solutions containing certain amino acids the respiratory rate was increased. They also studied the photosynthetic rates of excised leaves after varying periods of exposure to darkness, and found some correlation between the respiratory rates and the photosynthetic rate. GREGORY and RICHARDS (3) grew barley plants with various deficiencies of nutrient supply along with others with complete nutrient solution. They measured the respiratory rates of single leaves removed from the plants at various stages in growth and found that nitrogen deficiency had little or no effect on the CO_2 assimilation rate and a slightly depressing effect on the respiratory rate. BRIGGS (1), using excised leaves, also studied the effect of mineral deficiencies on the assimilation rate.

The purpose of the work here described was to determine the rates of photosynthesis and respiration of leaves attached to the plant, and of whole plants grown under different conditions of nitrogen nutrition, when such plants were subjected to various changes in the relative planes of nutrient supply. For example, if plants had been grown for a period with very low nitrate supply, so that they became relatively high in reserve carbohydrates, their photosynthetic and respiratory rates were studied first under the low plane of nitrate supply, then they were shifted to a high plane of supply and these same rates continuously determined. Similarly, tests were

made of plants on a high plane of nitrate supply and then shifted to a low one, with various intermediate conditions, as seemed desirable at any given time.

I. Experimental work on tomato

Methods and apparatus, etc.

Tomato plants were grown in the greenhouse in rich soil until they were about 6 inches high, at which time they were removed from the pots, the roots washed clean, and most of them clipped off close to the stem. The bases of the plants were then stuck 1–3 inches deep in clean moist quartz sand. New roots were soon developed. The nutrient solutions used throughout these experiments were the same as those described by Nightingale (**6**). The one containing nitrate was generally supplied for several days or longer following transplanting; but later all nitrates were omitted, and as a result the plants usually accumulated large reserves of storage carbohydrates as they continued to grow in the greenhouse.

At the beginning of each experiment six pots of plants were selected for uniformity and the relative vegetative or non-vegetative condition of the plants carefully noted. A general estimate of the reserves of nitrates and carbohydrates was determined by microchemical examination of the fresh tissue. The plants were then placed in a room which was held at constant humidity and temperature. Four 1000 watt tungsten bulbs placed at a distance of about 30 inches from the plants were used as the light source. An alternation of ten hours of exposure to light and fourteen hours in darkness was maintained throughout any given experiment.

For the detailed studies of carbon dioxide uptake and output, two leaves, one each from plants in separate pots, were selected. These will be referred to as the experimental leaves. They were green, approximately alike in appearance and area, and located as near the top of the plant as was possible, consistent with the ability to secure those which were fairly well matured and had attained practically full size. The leaf area was determined by printing an outline of the leaf on blueprint paper and later measuring the area by means of a planimeter. The light intensity was measured with a McBeth il-

luminometer. Adjustments were made so that the light intensity at the surface of each experimental leaf was the same at the beginning of each experiment.

The work with tomatoes was carried out in 1933. Since that time several changes have been made in the detail of the apparatus used. In its modified form it has been described by MITCHELL (4). The type of leaf chamber used, the method of sealing the plants into the chambers, the movement of gases through the chambers, and the methods of analysis of CO_2 are essentially the same as those described. In this early work, however, tungsten lamps were used as the light source. The concentration of CO_2 in the gas stream entering the leaf chamber was the same as in outside air. Under the light from tungsten bulbs there was a tendency for the leaf chambers to become heated, and in order to maintain the temperature inside the chamber equivalent to that outside it, the air in the chamber was cooled by passing it from the chamber by means of pumps (4) to coils in a cold water bath and then back to the chambers. There was no evidence in this work that the addition of nitrates to the plants used increased the rate of photosynthesis. This might be accounted for on the basis of some of the methods employed. The leaves inclosed in the gas chamber removed from 30 to 60 per cent of the CO_2 from the air stream passing over them and it may be that CO_2 was not present in sufficient quantities to permit an increase in the photosynthetic rate. It was also evident that the light source was not sufficiently intense nor of proper quality for growth and development of the plants, as shown by the fact that all the plants etiolated and lost some of their lower leaves. It was impossible to continue any given experiment for more than four or five days because the plants became so etiolated that they were no longer fit for use, or the leaves in the experimental chambers yellowed and died. It may be that if the proper light source were used and the CO_2 of the air stream in the chamber maintained at the same concentration as in atmospheric air, there would be an increase in the photosynthetic rate. Such a result was obtained, as is detailed later in the paper in the experiments on wheat.

EXPERIMENT I.—Six pots of plants were placed in the constant temperature room at 8 A.M. on July 28, 1933; two leaves were

selected on plants in different pots and sealed in the chambers. The circulation of gas through the chambers was started and the plants were illuminated until 4 P.M., which marked the close of the first light period. The plants were then in darkness for fourteen hours and this alternation of light and darkness continued throughout the experiment. Measurements of the CO_2 output of the experimental leaves were made throughout each period of darkness, records being taken at the end of every two hours. Nitrates were applied to three pots, including one of the pots with a plant bearing an experimental leaf, at the end of the second light period (in this case at 3 P.M., July 29).

The light intensity at the surface of each experimental leaf was 850 foot candles at the beginning of the experiment. At the end of the experiment it was 820 foot candles at the surface of the leaf on the plant which received no nitrate in the nutrient solution, and 850 foot candles at the surface of the other experimental leaf. The fall in light intensity in the former case was doubtless due to deterioration of the lamps used. The temperature of the room was 22° C. and the relative humidity was maintained at 75 per cent.

The plants when taken from the greenhouse were extremely high in reserve carbohydrates. All the cells of the mesophyll and palisade were filled with starch, which was abundant also throughout the parenchymatous tissues of the stem except at the very tip. The plants were hard and woody and showed an almost complete lack of nitrates. The changing composition of the plants was followed as closely as possible microchemically during the entire duration of the experiment. Those plants which were not inclosed in the small chamber and to which the nitrates were added remained high in carbohydrates, although they became slightly etiolated. The upper internodes lengthened, and growth at the tip took place rapidly. Yellowing and death of the lower leaves also occurred. The leaf inclosed in the chamber showed progressive yellowing from the margin of the leaflets inward during the course of the experiment and at the close was about to fall from the plant.

Those plants to which nitrates were added, when compared with those which received no nitrogen, appeared slightly greener, and both stem and leaves enlarged more than in the case of the minus

nitrogen plants. These also etiolated somewhat and lost their lower leaves. In four to six hours after nitrates had been added tests for nitrates were positive well up in the plants, and in twenty-four hours nitrates were in abundance throughout. Nitrites became apparent about two hours after the nitrates were detectable. After fourteen hours the plus nitrate leaf was noticeably greener than the minus nitrogen one, and had enlarged appreciably.

TABLE I

EXPERIMENT I: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO_2 RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)			
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD	4TH DARK PERIOD
	PLUS NITRATE			
1	167	84	171	.....
2	119	84	163	143
3	116	92	163	86
4	.....	95	.....	86
5	99	.....	155	86
6	73	109	131	73
	MINUS NITRATE			
1	203	120	121	.....
2	155	104	112	73
3	131	112	118	86
4	129	99	111	86
5	114	94	104	86
6	114	87	104	87

The results of the respiratory measurements are given in table I. It is obvious that following the addition of nitrates, the respiration rate is appreciably increased.

EXPERIMENT II.—This experiment was begun in the constant temperature room on August 2, 1933. The same details of procedure were followed as before. When placed in the room the plants were nitrate-free but were slightly lower in reserve carbohydrates than those of the previous lot. They appeared slightly greener and the

stem tips were softer. They were high in reserve carbohydrate, however, and behaved very similarly to those of experiment I. During the course of the experiment they etiolated somewhat and lost their lower leaves. The experimental leaf of the plant to which no nitrates had been added became yellow as before and was ready to fall from the plant at the close of the experiment. Those plants to which

TABLE II

EXPERIMENT II: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO_2 RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)		
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD
	PLUS NITRATE		
1	202	144	199
2	148	125	165
3	148	114	165
4	119	119	153
5	125	94	165
6	114	95	153
	MINUS NITRATE		
1	254	188	126
2	151	126	108
3	126	126	100
4	126	107	108
5	125	115	100
6	123	126	111

nitrates were added became noticeably greener and enlarged considerably.

Nitrates in slight amounts made their appearance in the upper parts of the plants in twelve hours after their application. Nitrites were evident several hours after the nitrates appeared in the tops, but were present only in very small amounts.

The results of the respiratory measurements are given in table II. In general the plants responded more slowly to nitrate application

than did the plants of the previous experiment. As compared with the results of experiment I, there was less increase in the rate of respiration after the application of nitrates and the response was delayed for a longer period.

EXPERIMENT III.—The plants were removed to the constant temperature room on August 8, 1933 at 8 A.M. and the measurements

TABLE III

EXPERIMENT III: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO_2 RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)		
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD
	PLUS NITRATE		
1	182	.....	200
2	133	137	168
3	139	128	159
4	139	111	168
5	135	106	166
6	116	90	164
	MINUS NITRATE		
1	190	143	.....
2	151	136	78
3	147	133	102
4	149	88	89
5	147	.....	71
6	125	100	.....

started at 4 P.M. as before. The procedure followed was the same as that used in the previous two experiments. The plants used might be described as being intermediate in condition and response between the plants of the first and the second experiments. They were slightly taller than those of the previous two lots, but the number and size of the leaves were about the same. They were not so high in reserve carbohydrates as the first lot of plants, but higher than the second. Upon the application of nitrates the plants turned

greener and enlarged as before. Nitrates were present in small amounts after six to eight hours, and nitrites appeared a few hours later.

The results of the respiratory measurements are given in table III. In general the results occupy an intermediate position between those obtained in the first and second experiments.

TABLE IV

EXPERIMENT IV: RESPIRATION IN TOMATO PLANT

INTERVAL OF READING (EACH OF 2 HOURS' DURATION)	CO_2 RESPIRED PER SQ. METER OF LEAF AREA PER HOUR (MG.)		
	1ST DARK PERIOD	2ND DARK PERIOD	3RD DARK PERIOD
	PLUS NITRATE		
1	63	.....	162
2	60	64	173
3	55	68	184
4	48	64	.....
5	52	75	160
6	32	72	139
	MINUS NITRATE		
1	81	.....	18
2	81	37	18
3	67	24	10
4	67	29	.....
5	70	32	10
6	56	36	0

EXPERIMENT IV.—Except for the fact that they were slightly larger, contained more lignified tissue, and had a slightly hollow stem, the plants used in this experiment were similar to those used in the first experiment. They were extremely high in reserve carbohydrates; and as in the first experiment, all of the mesophyll and palisade cells of the leaves were filled with starch which was abundant also throughout the parenchymatous tissues of the stem except at the very tip. No reserves of nitrates were apparent.

The plants were placed in the constant temperature room on August 15, 1933 and treated as usual. Nitrates were present throughout the plants within three hours after their application in the nutrient solution, and nitrites appeared two hours later. The leaves of the plants to which nitrates were added became greener than the others within eight to ten hours after the application of nitrates.

Results of the gas analyses are given in table IV. The results are similar to those in experiment I except that the increase in rate of respiration is greater.

SUMMARY

On the basis of the foregoing data it is evident that the composition of any given plant is in a marked degree correlated with its respiratory activity. Thus tomato plants very high in carbohydrates but low in their nitrate or soluble nitrogen content may have a relatively low respiratory rate, whereas one which is higher in its nitrate content will have an appreciably higher one. Or any given plant high in its carbohydrate reserves will have its respiratory rate increased if nitrates are supplied to it in abundance, other conditions remaining the same. Although all the plants used in the various experiments were very high in reserve carbohydrates, a relatively slight change in the amount of this reserve had a great effect upon the time required for nitrates to make their appearance in the tops of the plants after they had been applied in the nutrient solution. In every case the addition of nitrates to plants high in reserve carbohydrates caused an increase in respiration, the greater such reserve the more nearly immediate the response and the greater in degree. In the case of plants extremely high in reserve carbohydrates the response was almost immediate and very marked. In the first and last experiments respiration of the plants to which nitrates had been added increased to three times that of the plant which received no nitrate. In the case of plants slightly lower in reserve carbohydrates the response was not so great, nor did it occur at once. In the second experiment a response was noticeable only after twenty-four hours, and the increase in respiration was about 100 per cent. In the third experiment the response became evident in twenty-four hours, and the total increase was 120 per cent. In each case the increase in rate

of respiration seemed to take place subsequent to the appearance of nitrates in the top of the plant; in no instance did it occur prior to such appearance, whatever the length of time elapsing after their application in the nutrient solution.

II. Experimental work on wheat

It was desired to supplement the work on tomatoes, since the measurements were made on single leaves instead of on entire plants. Also it was apparent that the plants would not accumulate reserves of carbohydrates nor maintain those reserves already present when they were placed under Mazda lights. In the work on wheat it was found possible under artificial illumination to grow plants which would continue to increase in size and in dry weight. By varying the nitrogen supply in the nutrient solution, plants either very high or very low in reserve carbohydrates, as was desired at any particular time, were obtainable. It was possible also to obtain separate quantitative measurements on the respiration of roots and of tops and also measurements of the CO_2 assimilation of the entire tops.

The apparatus finally devised and used in this work is that described in detail by MITCHELL (4). That part of the apparatus described for experimentation with entire plants was designed for these experiments.

Wheat grains of the Marquis variety, secured from Corvallis, Oregon, were sterilized in 0.25 per cent uspulun for five minutes and planted in sterilized quartz sand. In each experiment 50 grains were planted per pot. Six-inch clay pots and pyrex beakers of 1500 cc. capacity and with necessary outlets were used as containers. These were placed in the constant temperature room, watered with distilled water for the first week, and exposed to a light intensity of about 300 foot candles. At the end of the first week the pots containing the plants were placed in a pan of distilled water so that the water level reached the upper surface of the sand in the pot, and a little of the sand around the base of the seedlings was washed away. The ungerminated grains were picked out. All seedlings in excess of fifteen per pot were removed in entirety, care being taken that those remaining be as uniform in size as possible.

The pots were then placed about 30 inches from the light in posi-

tions such that the plants in each pot were exposed to an intensity of about 800 foot candles for a period of twelve hours. The light was then turned off and the plants were in darkness for the remaining twelve hours of each day.

The nutrient solutions employed throughout these experiments were the same as those used by NIGHTINGALE (**6**). One of these contained no nitrate, the other contained nitrate in abundance. For four days following the time when the cultures were placed in full light they were supplied with the nutrient solution containing no nitrate, diluted one-half with distilled water. For at least two days more all the cultures received the same solution, undiluted. Subsequent treatment of the plants varied in the several experiments conducted. In all of them, however, the plants were sealed in the chambers at twelve to fourteen days after the sowing of the grains. After sealing in the chambers all the cultures were supplied with minus nitrate solution for two days more. Then, commencing at the end of the light period of the third day, half of the plants received the same minus nitrate solution and half the plus nitrate solution. This treatment was continued throughout any given experiment.

During the first two days in the chambers, readings were made of the CO_2 uptake during the last four hours of the light period, and CO_2 output during the first four hours of the dark period. At the end of the light period of the third day the plus nitrate nutrient solution was applied to one-half of the cultures, and readings on CO_2 output of both the plus and minus nitrate cultures made for the following four-hour dark period. Subsequent readings of uptake and output were made for such periods and at such times as already stated for the first two days the plants were in the chambers.

It was found that essentially the same values were obtained for the uptake and output over a given time interval, whether the readings were made at the beginning or the end of a given light or dark period. For convenience in making measurements and in recording results, only readings for the last four hours of the light period and the first four hours of the dark period of each day are given.

In each experiment thirty groups of plants were used. Six of these were the pyrex beakers mentioned, and twenty-four were the 6-inch clay pots. The plants in the six beakers were used for the measure-

ments of CO_2 uptake and output. At the time when nitrates were added to some of the plants, those in eight of the clay pots were harvested and constituted the initial sample. Such plants, of course, had received no nitrate. Eight of the remaining clay pots and three

TABLE V

EXPERIMENT V: QUANTITATIVE DETERMINATIONS OF CO_2 UPTAKE AND OUTPUT BY YOUNG WHEAT PLANTS

Age of culture in days	CO_2 assimilated per 100 plants during 4-hour interval of period of exposure to light (mg.)	CO_2 liberated per 100 plants during 4-hour interval of period in darkness (mg.)		CO_2 assimilated per 100 plants during 4-hour interval of period of exposure to light (mg.)	CO_2 liberated per 100 plants during 4-hour interval of period in darkness (mg.)	
		Roots	Tops		Roots	Tops
	Receiving no nitrogen in nutrient solution					
14	186	32.4	15.2	164	33.2	11.9
15	186	29.8	15.5	152	30.6	14.9
16	186	.....	.....	154	.....	.....
	After addition of nitrates to nutrient solution supplied to some of them					
	Plus nitrate			Minus nitrate		
16	.....	41.7	15.5	.....	29.8	13.6
17	221	42.1	24.2	133	29.6	13.7
18	290	35.6	18.0	140	30.0	14.0
19	.....	21.0	15.2	.....	29.8	14.1
20	310	31.9	21.3	154	.....	.....
21	290	.....	.....	140	30.8	11.1
22	279	35.1	21.5	133	.....	.....
23	279	.....	.....	128	31.9	10.4
24	.....	39.0	22.3	.....	.....	.....
25	234	.....	.....	138	30.1	8.9
26	234	41.0	23.8	140	.....	.....
27	.....	.....	.....	.....	30.4	9.1
28	226	42.8	29.0	168	29.3	8.7

of the beakers were then supplied with nitrates in the nutrient solution and the others were continued with the minus nitrogen nutrient solution. At the end of the experiment all the plants in the clay pots were harvested and constituted the final sample.

DISCUSSION OF RESULTS

The results of three separate experiments are given here, since they are representative of several which show the same results. The first experiment extended from the time the plants were fourteen days old until they were twenty-eight days old. At the age of fourteen days none of the plants showed definite visible signs of nitrogen deficiency except that they were a light green. At the end of the experiment, however, those plants which had been supplied with no nitrogen in the nutrient solution showed definite injury. The lower leaf was usually yellow throughout its length and nearly dead; often the second leaf was dead at the tip and yellow nearly half the distance toward the base of the leaf. The rest of this leaf and the rest of the leaves were a yellowish green in color. Those plants to which nitrates had been added were a deep green except for the lowest leaf which often was partially dead. The other leaves were a fresh green color and continued to increase rapidly in size, more than doubling in area by the end of the experiment. The significant data are given in tables V–VI.

TABLE VI

EXPERIMENT V

	DRY WEIGHT PER 100 PLANTS (GM.)			FRESH WEIGHT PER 100 PLANTS (GM.)		LEAF AREA PER 100 PLANTS (SQ. DM.)
	ROOT	TOP	TOTAL	ROOTS	TOPS	
Initial sample.....	1.400	1.627	3.027	20.90	12.1	5.00
Plus nitrate plants 14 days later.....	1.803	4.352	6.155	37.1	34.9	13.06
Minus nitrate plants 14 days later.....	2.430	2.168	4.598	47.5	16.4	6.04

A second experiment of the same general nature as the first was also performed. All conditions were kept as nearly the same as possible, except that readings on uptake and output were begun when the plants were twelve days old instead of fourteen. This was in order that the plants might not have undergone as much injury from nitrogen deficiency as under the first experiment. The data are given in tables VII–VIII.

A third experiment was carried out to eliminate some of the variations apparent in the preceding two experiments and to obtain data concerning the changes in root volume and root area. The root volume was obtained by placing the clean fresh roots in water and

TABLE VII

EXPERIMENT VI: QUANTITATIVE DETERMINATIONS OF CO_2 UPTAKE AND OUTPUT BY YOUNG WHEAT PLANTS

Age of culture in days	CO_2 assimilated per 100 plants during 4-hour interval of period of exposure to light (mg.)	CO_2 liberated per 100 plants during 4-hour interval of period in darkness (mg.)		CO_2 assimilated per 100 plants during 4-hour interval of period of exposure to light (mg.)	CO_2 liberated per 100 plants during 4-hour interval of period in darkness (mg.)	
		Roots	Tops		Roots	Tops
	Receiving no nitrogen in nutrient solution					
12	178	32.5	20.5	178	31.9	14.6
13	189	.....	.....	178	.....	.....
	After addition of nitrates to nutrient solution supplied to some of them					
	Plus nitrate			Minus nitrate		
13	.....	51.9	17.5	.....	31.9	14.9
14	201	40.4	17.3	165	33.5	14.3
15	247	32.4	19.1	161	34.6	13.3
16	258	34.6	20.5	149	.....	.....
17	266	38.6	24.5	160	31.4	14.1
18	.....	.....	.....	.....	32.4	12.8
19	279	38.6	25.2	146	.....	.....
20	.....	41.2	26.8	.....	31.9	12.2
21	247	.....	.....	160	.....	.....
22	237	45.2	31.1	145	31.9	12.8
23	.....	.....	.....	.....	.....	.....
24	235	47.9	31.9	142	30.6	8.5

measuring the volume of the displaced liquid. The root area was an approximation calculated from the area covered by the projection of the root system on to a plane surface. The latter area was obtained with an apparatus described by MITCHELL (5). The clean fresh roots were spread out on a plate glass and the area covered

by their shadows measured as described. This area was multiplied by 3.14 to give root area. All conditions were kept as nearly the same as those of the first experiment as possible, except that the plants were watered with minus nitrogen nutrient solution diluted one to ten with distilled water during the first week. Screens were placed behind the chambers in such a manner that some of the light passing through or around them might be reflected back, thus giving a more

TABLE VIII

EXPERIMENT VI

	DRY WEIGHT PER 100 PLANTS (GM.)			FRESH WEIGHT PER 100 PLANTS (GM.)		LEAF AREA PER 100 PLANTS (SQ. DM.)
	ROOT	TOP	TOTAL	ROOTS	TOPS	
Initial sample.....	1.160	1.488	2.648	20.8	11.66	4.47
Plus nitrate plants 12 days later.....	1.380	3.540	4.920	35.0	31.8	10.82
Minus nitrate plants 12 days later.....	2.00	2.04	4.04	45.0	15.8	4.86

nearly uniform lighting of the entire chamber and of the plants inside it. The significant data are given in tables IX and X.

It will be obvious at once that there was some fluctuation in the readings, each of which is the average of three separate pots of plants (45 plants in all), but there are definite and marked trends, which are evident in all the experiments. It is not yet possible to account precisely for some of the variations in CO_2 uptake and output. They may have been due to minor fluctuations in the relative humidity or to other seemingly minor variations in environmental conditions. Great care was taken to keep these factors as nearly uniform as possible, but at times they were not absolutely constant. As already stated, screens were placed behind the plants in the last experiment in order to obtain a more nearly uniform lighting of the entire chamber and of the plants. This was done because in the previous experiments a slight fog had accumulated on one side of the chambers and it was thought that this might reflect varying amounts of light according to its density. The readings in the last experiment are slightly more uniform than those of the previous

experiments. All the experiments agree in their general trends and the trends shown are definite and of sufficient magnitude to remain unobscured and valid in spite of the variations shown.

TABLE IX

EXPERIMENT VII: QUANTITATIVE DETERMINATIONS OF CO_2 UPTAKE AND OUTPUT BY YOUNG WHEAT PLANTS

Age of culture in days	CO_2 assimilated per 100 plants during 4-hour interval of period of exposure to light (mg.)	CO_2 liberated per 100 plants during 4-hour interval of period in darkness (mg.)		CO_2 assimilated per 100 plants during 4-hour interval of period of exposure to light (mg.)	CO_2 liberated per 100 plants during 4-hour interval of period in darkness (mg.)	
		Roots	Tops		Roots	Tops
	Receiving no nitrogen in nutrient solution					
14	167	29.6	15.1	173	30.9	14.0
15	175	29.0	14.7	172	29.8	13.8
16	178	.....	.....	168	.....	.....
	After addition of nitrates to nutrient solution supplied to some of them					
	Plus nitrate			Minus nitrate		
16	.....	40.0	16.1	.....	29.6	13.5
17	219	45.0	16.6	170	30.1	14.1
18	220	30.9	15.8	166	30.0	13.7
19	260	32.1	17.1	166	31.2	13.3
20	280	34.2	17.0	176	30.8	13.5
21	250	34.0	18.6	174	29.9	12.8
22	245	36.0	20.5	168	30.4	13.1
23	260	36.1	23.9	176	29.8	12.5
24	260	37.8	25.0	168	29.8	11.8
25	262	39.1	25.0	176	30.7	10.8
26	.....	41.6	26.2	.....	30.6	10.6
27	256	42.7	26.9	167	31.2	10.1
28	250	45.3	28.7	170	30.5	9.2
29	245	50.8	30.9	172	30.2	8.7
30	238	.....	.....	174	.....	.....
31	240	52.9	32.4	170	31.8	8.8

CULTURES TO WHICH NO NITRATE WAS SUPPLIED.—Perhaps the most striking result is the fact that the minus nitrate plants, although part of the leaves died and those that remained alive became

yellow to yellowish green and increased but little in size, yet were able to assimilate relatively large quantities of CO_2. That there is not an error of measurement involved is confirmed by the figures on the dry weight of these plants. These showed an increase comparable with the amount of CO_2 absorbed over the amount lost in respiration. This must mean, therefore, that on the basis of unit area of the leaf the rate of carbon fixation has undergone practically no decrease during the experiment. It is not possible to say with precision just how many living active cells capable of photosynthesis there may

TABLE X

EXPERIMENT VII

	DRY WEIGHT PER 100 PLANTS (GM.)			FRESH WEIGHT PER 100 PLANTS (GM.)		LEAF AREA PER 100 PLANTS (SQ. DM.)	ROOT AREA PER 100 PLANTS (SQ. DM.)	ROOT VOLUME PER 100 PLANTS (CC.)
	ROOT	TOP	TOTAL	ROOTS	TOPS			
Initial sample	1.20	1.60	2.80	20.5	12.05	4.17	12.62	27.7
Plus NO_3 plants 16 days later	1.87	5.28	7.15	36.3	45.32	14.77	19.77	40.1
Minus nitrate plants 16 days later	2.40	2.48	4.88	46.04	15.5	4.51	21.54	34.5

have been nor what their exact volume was, but that those which were capable of functioning continued to do so at a fairly rapid rate there is no doubt. The relatively large capacity of these cells to fix the quantity which they did may well furnish a definite basis for understanding why plants which are small because of nitrogen starvation are still able to accumulate large amounts of carbohydrate or carbohydrate derivatives, either as reserve foods or structural materials. It may also aid in an interpretation of the behavior of some species when subjected to short photoperiods.

The total amount of CO_2 output of the tops during the periods of the experiment showed a quantitative decrease. This is in accord with the results of GREGORY and RICHARDS (3). Thus the rate of respiration per unit of area would at first seem to be markedly less, but actually may not have been so, since some of the apparently liv-

ing tissues measured may actually have been dead. Again the results are of such nature that valid deductions are possible.

In contrast to the relatively small increase in the tops of these minus nitrate plants, the roots gained appreciably in volume, area, and more than 100 per cent in dry weight. Since the total amount of CO_2 given off remained about the same despite the increase in volume of the root and since there was virtually no dead tissue present, the respiratory rate showed an actual decrease. It is apparent, therefore, that the elaborated materials from the tops were in this case used in extension of the root system and the building up of a dry weight reserve. This is in marked contrast to the roots from the plus nitrate cultures, as will be discussed later.

CULTURES TO WHICH NITRATE WAS SUPPLIED.—The tops of these plants began to increase markedly in size after the application of nitrate. They were a fresh bright green and by the end of the experiment had more than doubled in dry weight. The volume of CO_2 absorbed increased markedly during the first five days, then declined somewhat despite the continued increase in leaf area, and finally leveled off to an amount slightly in excess of that before the nitrate was applied. Thus it is obvious that the rate of photosynthesis per unit of leaf area at first markedly increased and then later actually decreased. It was thought that most of the leaves which expanded during the experiment might already have been differentiated when nitrate was added so that there was little or no increase in the number of stomata operative. That this was not true was shown by stomatal counts. It may be that this decrease in the rate of CO_2 uptake is associated with the increasing age of the plants. GREGORY and RICHARDS (3) claim that in barley there is a decrease in the photosynthetic rate of all of the leaves as the plant grows older. There was no such decrease in the minus nitrate plants, however. It is possible that at the beginning of the period there was a metabolizable carbohydrate reserve in the leaves which soon became used up in expansion of the volume of leaf and that the newer chemical relations set up within the leaf were not favorable to increased photosynthesis. There was at all times an abundance of CO_2 in the surrounding atmosphere so that it did not become a limiting factor. The results, which were obtained repeatedly in these experi-

ments, were not wholly in accord with what might have been expected on the basis of many other results already recorded in the literature. This difference, however, makes the results obtained all the more interesting, for they certainly call for a further chemical investigation of leaves in relation to their capacity for CO_2 uptake and fixation.

In general the tops of the plants showed a gradual rise in CO_2 output as the leaves expanded. Except in one instance, there was no abrupt rise and fall, and in that instance there was a high initial carbohydrate content of the leaves. On the basis of unit of leaf area, the respiration rate remained about the same or even slightly decreased, despite the increased expansion rate of the leaves, which might have been expected to be accompanied by a considerably increased respiration rate. It seems obvious that most of the products of photosynthesis were used in the manufacture of the tissues of the leaf, as reflected in the increased dry weight.

In contrast to the roots of the minus nitrate cultures, those of the plus nitrate cultures gained little in dry weight over the initial cultures. This is in harmony with many findings relative to root-top ratios when plants are liberally supplied with nitrates, and especially if reserve carbohydrates are small in quantity or conditions for their synthesis or translocation limited. It is interesting to note that the volume of the root system of these cultures (table X) increased appreciably and more in relation to root area than did the minus nitrate cultures. This might be expected since the individual roots were much greater in diameter and more succulent than those of the minus nitrate plants. After the application of nitrates the CO_2 output in these experiments rose sharply and increased markedly, such increase being followed presently by an abrupt drop to an amount about equal to that of the control plants, and then another slight increase. Since there was some slight increase in the volume of the roots during the experiment, the rate of respiration per unit of volume at the end of the experiment was about equal to that of the initial plants.

Recalling the fact that the roots of the minus nitrate plants, although showing a marked increase in their carbohydrate content, did not show a marked increase in respiration rate, it is safe to say

that the presence of carbohydrates alone in and of themselves do not necessarily mean an increased respiration rate. But if nitrates are added to the nutrient solution, then it is possible that these may become elaborated into amino acids, proteins, and the like, which may have a marked effect on the respiration rate, as SPOEHR'S results have previously shown. Thus there could readily be a rapid rise of respiration rate in the case of the roots in these experiments. Similarly such a rise might be expected to be followed by a fall unless the reserve of carbohydrates were very large or unless there was a comparatively large amount of carbohydrates constantly being translocated to the roots. This latter condition apparently did not prevail, such carbohydrates as were synthesized being utilized largely in top expansion. Finally, although nitrates were abundant even after the first brief period, the carbohydrate supply in the roots may well have become a limiting factor in the respiratory rate.

A condition very similar to that shown by these plus nitrate roots was clearly indicated by the tomato leaves, which contained a large supply of carbohydrates at the time of application of the nitrates, and also by the tops in one of the experiments with wheat, when such tops contained a fairly large initial content of carbohydrates. The abrupt rise of respiration rate was not observed in the tops of those plants which did not have such a reserve, even though nitrates were added, as has already been stated.

Comparison of work on tomato and wheat

With respect to respiration, the same general results were secured with both plants. There can be no doubt concerning the resultant marked increase in the respiration rate by the application of nitrate to the plant provided there is an initial carbohydrate reserve. The rate is roughly proportional to the amount of such carbohydrate content. This is true whether the organ experimented upon is leaf or root.

It is equally clear that a relatively high photosynthetic rate may be maintained by leaves low in soluble forms of nitrogen, relatively low in content of chlorophyll, and relatively high in carbohydrate content of the cells. These findings should be accompanied by critical qualitative and quantitative chemical analyses at the time

the demonstrations are made. Detailed analyses are not available for the experiments presented, but roughly quantitative measurements of some of the compounds were made, and have already been commented upon.

The apparatus as herein described is adequate for the determination of CO_2 output and uptake by plants. It is delicate and requires care in its operation, but the results obtained with it under uniform conditions may be relied upon. The next step is to correlate these findings with the chemical composition of the organs being studied and with critical quantitative measurements of areas, volumes, and the like, so that the rates of the processes of photosynthesis and respiration may be precisely determined. This is particularly necessary because the present results, found over and over again in these experiments, are not completely in accord with some previous results as found in the literature and more or less taken for granted as standard and final. The whole subject is still open for further critical experimentation and a revaluation of the data already recorded.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C.

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GRASS SEEDLING ANATOMY: THE FIRST INTERNODE OF AVENA AND TRITICUM

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(WITH SIX FIGURES)

Introduction

Our knowledge of the anatomy of grass seedlings has developed chiefly through studies of homologies that have been made during the past 100 years. In the last decade a developmental approach has been made in such studies, particularly with reference to the coleoptile of the *Avena* seedling. With the extensive use of this plant for quantitative work in hormone research, it seems especially appropriate at this time to clear up some of the problems connected with the vascular relationships of the cotyledon and coleoptile to the seedling axis. The movement of hormones or their precursors from the cotyledon into the seedling axis, and subsequently into the coleoptile, depends largely upon vascular pathways; similarly, the movement of water from the seedling roots to the cotyledon, coleoptile, and later leaves.

We were prompted to re-examine the vascular relationships of seedling organs of oats and wheat because the epiblast of the seedling in the Gramineae has been interpreted once more as a second cotyledon (**7**), an interpretation which would necessitate classifying the epiblast-bearing grasses with dicotyledonous plants. There is nothing else about the structure of grasses supporting such a drastic interpretation, or change in their taxonomic position. It is more probable that the grass seedling is in close structural harmony with the seedlings of related monocotyledonous groups.

The external morphology of the embryo and seedling of *Avena* and *Triticum* is so well known that only the briefest reference to it need be made here. The cotyledon consists solely of the "scutellum," and

[1] This investigation was undertaken during my tenure of the Rose Sidgewick Fellowship. It is a pleasure to acknowledge my thanks to the American Association of University Women and to Connecticut College for facilities for study.

its vascular system has been described (2). In certain genera such as *Avena* there is an outgrowth present on the face of the scutellum. This is called the "ventral scale," and is a curved ridge which extends downward from above the tip of the coleoptile, partially surrounding the base of the embryonic axis. It is most marked in genera possessing an epiblast. The latter is a parenchymatous, nonvascular structure on the axis opposite the scutellum. The internal root-stem transition occurs about the level at which the epiblast is borne. The ventral scale and epiblast are together interpreted (3) as the ligule of the cotyledon. Such a ligule occurs as a complete structure in the "ligulate type" of monocotyledonous seedling. The level of attachment of the scutellum to the axis marks the first, or cotyledonary node of the plant.

The coleoptile which sheathes the embryonic leaves is interpreted as the first leaf of the plant above the cotyledon, and the level of its attachment to the axis marks the *coleoptilar node*. Structurally the coleoptile is similar in the two genera. It differs in two respects from the later leaves: it is not differentiated into sheath and blade and it possesses only two veins, one on either side. Its simplicity of structure in no way invalidates it as the leaf next above the cotyledon, an interpretation based on its vascular relationship to the axis. Since the coleoptile is the second leaf of the plant (the cotyledon being the first), it follows that the level of coleoptilar divergence from the axis marks the upper limit of the first internode; that is, it is the second node.

The behavior of the cotyledonary bundle upon entering the axis in the seedling is mainly dependent on the location (in the mature embryo) of the meristematic zone which is responsible for the initial elongation of the first internode (2). In *Triticum*, elongation takes place principally in the second internode and is negligible in the first internode. The scutellar trace is therefore relatively unaffected by the actively elongating region of the axis. The corresponding meristematic zone in *Avena* surrounds the scutellar bundle; and when elongation occurs at germination, the scutellar bundle is laid down in an upward direction in the cortex, parallel to the stele.

This study is concerned mainly with the anatomy of the axis between the first (cotyledonary) and second (coleoptilar) nodes; that is,

the first internode and the transition plate at which root structure begins. Further evidence is offered to support the earlier interpretation that this interval is the first internode of the seedling, and thus aid in bringing the seedling of the grasses into morphological harmony with those of other monocotyledons.

Investigation

Anatomy of axis between transition plate[2] and level of coleoptilar divergence

Triticum vulgare

The axis between the transition plate and the level of coleoptilar divergence in *Triticum* is exceedingly short (2). In a two-weeks-old seedling the vascular make-up of the stele at the level of coleoptilar divergence (figs. 1, 2) consists of: (1) the two coleoptile bundles, (2) the traces of the next leaf above the coleoptile (a midrib and three pairs of lateral bundles), and (3) the traces of the third leaf (midrib and a pair of lateral bundles). Slight anastomoses at this level do not affect to any extent the stelar anatomy.

The vascular elements of the axis between the transition plate and the coleoptilar node may, for purely descriptive purposes, be resolved into four groups. In no way does this represent the ontogenetic development.

1. A bundle connects the midrib of the second leaf to the transition plate (the nodal plate of the cotyledon). It has no connection with any other leaf trace (fig. 2 *A–E*).

2. The scutellar, coleoptilar, and second leaf traces are connected on the scutellar side of the axis with a "common bundle"; that is, a bundle which passes through the stem for a greater or less distance (fig. 1) but terminates as a leaf trace or traces (5). Each median lateral bundle of the second leaf is united with the neighboring coleoptilar bundle. The bundles which thereby result are joined by the scutellum trace, making a common bundle which extends approximately 0.5 mm. to the nodal plate of the cotyledon, where it is united with the bundle from the midrib of the second leaf. The re-

[2] The terms transition plate, cotyledonary plate, and nodal plate of the cotyledon are equally useful and interchangeable.

mainder of the system is symmetrical about the plane in which these two bundles lie.

3. A third system involves the remaining two pairs of lateral bundles of the second leaf and the bundles from the third leaf (figs. 1, 2). The midrib of the third leaf and the outer lateral bundles of

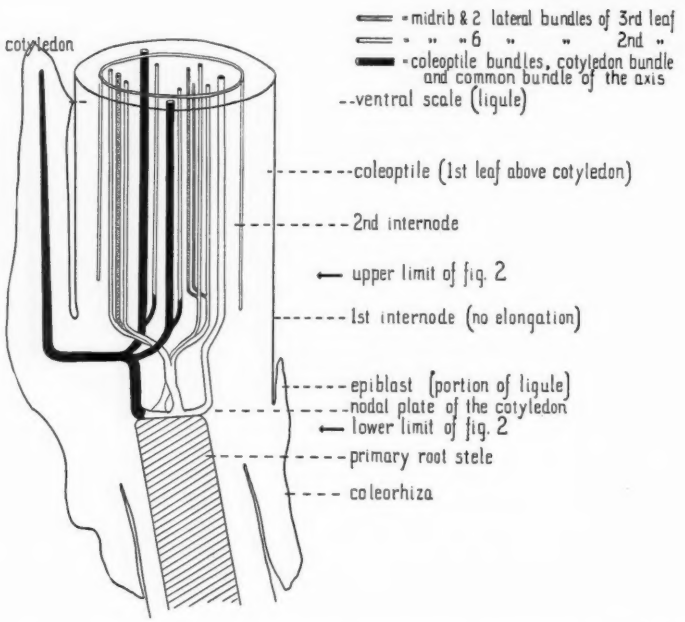

= midrib & 2 lateral bundles of 3rd leaf
= " " " 6 " " 2nd "
= coleoptile bundles, cotyledon bundle and common bundle of the axis
cotyledon
--ventral scale (ligule)
coleoptile (1st leaf above cotyledon)
2nd internode
upper limit of fig. 2
1st internode (no elongation)
epiblast (portion of ligule)
nodal plate of the cotyledon
lower limit of fig. 2
primary root stele
coleorhiza

FIG. 1. *Triticum vulgare:* diagrammatic reconstruction of portion of seedling showing principal structures present, and vascular inter-relationships of cotyledon with axis, coleoptile (first leaf above the cotyledon), and first characteristic foliage leaf. Nodal plate of the cotyledon is main level at which root-stem transition takes place. First internode is also in part a transition region. From levels of divergence of cotyledon and coleoptile, it will be understood that the first internode in *Triticum* does not elongate.

the second leaf extend closely parallel. The resulting plexus of these three closely parallel amphivasal bundles connects by two small strands (of half a dozen elements) to the common bundle at the place where it branches to the scutellum and upper leaves (fig. 2 *B*).

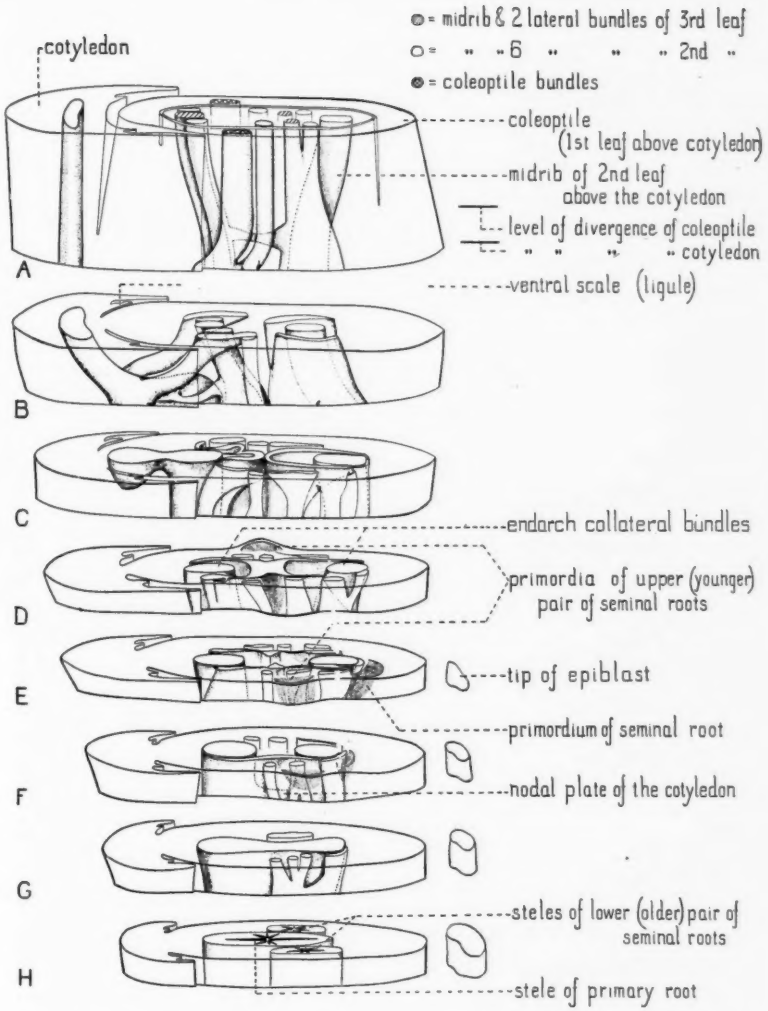


FIG. 2. *Triticum vulgare:* detailed course of vascular bundles and scattered vascular elements in portion of seedling, upper and lower limits of which are indicated in fig. 1.

Each lateral bundle from the third leaf fuses with the neighboring inner lateral bundle of the second leaf (fig. 2 *A*). The resulting bundles join the plexus just referred to at or slightly below the level where the cotyledonary trace enters the axis (fig. 2 *B*, *C*). The vascular network so formed extends through approximately 0.3 mm., then divides into two thin plates of tissue, lateral to the two main bundles of the axis; these inclose a small pith (fig. 2 *E*, *F*). A little lower down the vascular network consists of the anastomosing tissue of the transition plate (fig. 2 *F*, *G*).

4. A pair of roots occurs at the transition plate in a plane parallel to the cotyledon. They are not always at exactly the same level, but the differences are of little importance (fig. 2 *D*, *E*). A third root may appear near the epiblast. Since the initials of these roots are present in the embryo, they have been termed seminal roots (**8**). Like the primary root, each has seven or eight xylem rays in the stele. Where only two arise their relation to the seedling axis is similar. The stele of each seminal root divides; half the elements extend upward, finally uniting with others to form the midrib of the second leaf. The other half forms a trace which bifurcates; one branch joins the inner lateral trace of the second leaf, the other connects with the middle lateral bundle of the second leaf immediately above its fusion with the coleoptilar bundle. The two seminal roots therefore connect with the lateral bundles of the second leaf, to a more limited extent with its midrib, and indirectly with the lateral bundles of the third leaf.

A second pair of seminal roots arises a little above but in the same plane as the older set, but for simplification they were omitted in figure 2. This level corresponds roughly with that at which the scutellum trace enters the axis. They connect with the same vascular strands as the first or lower pair of seminal roots.

Avena sativa

In the young seedling it is this portion of the axis between the cotyledon and the coleoptile which is the principal organ of elongation. It raises the second node and higher nodes (the ultimate aerial portion of the plant) to the surface of the soil. The cotyledonary trace is so located with respect to the meristematic zone that it

differentiates upward in the cortex of the elongating axis (fig. 3). In the mature seedling it appears in a transection of the first internode merely as a cortical bundle.

Just below the level of coleoptilar divergence (fig. 4 *A*) the vascular system of the axis consists of the following: (1) the two coleoptile bundles, (2) the midrib and three pairs of lateral bundles of the second leaf, and (3) the midrib and a pair of lateral bundles of the third leaf. The stelar anatomy is on the same general plan as that of *Triticum vulgare*, and will be described in a similar manner:

1. A bundle extends unchanged from the midrib of the second leaf to the transition plate (figs. 3, 4).

2. Each coleoptilar bundle fuses with the outer and median lateral bundles of the second leaf which lie right and left of it and then joins with the scutellar trace to form a common bundle (figs. 3, 4 *A*). The latter extends downward in the stele parallel to the cortical scutellar bundle. At the transition plate connection is made between the common bundle and the bundle from the midrib of the second leaf (fig. 3).

3. Each inner lateral bundle of the second leaf is united with the neighboring lateral bundle of the third leaf and then with half the midrib of the third leaf (fig. 4 *A*). The resulting bundles extend downward into a meristematic zone (the equivalent level of the "plexus" referred to in *Triticum*), where they lose their identity. It is in this meristematic zone that new cells arise, cells which upon subsequent enlargement are responsible for the elongation of the first internode. In the two-weeks-old seedlings examined, certain highly lignified xylem elements may be traced practically through the meristematic zone. These connect the inner lateral bundles of the second leaf (and associated strands of the third leaf) with elements which extend to the transition plate (fig. 4 *A–E*). The stelar arrangement appears the same in transection from approximately the level of the meristematic zone to the transition plate (figs. 3, 4).

4. A pair of seminal roots is borne at the scutellar node. The vascular connection of these roots is to the elements which lie between the two main endarch bundles (not illustrated, but occurring a little above the level indicated as the nodal plate of the cotyledon in fig. 4 *E*). When three seminal roots occur at this level, the third

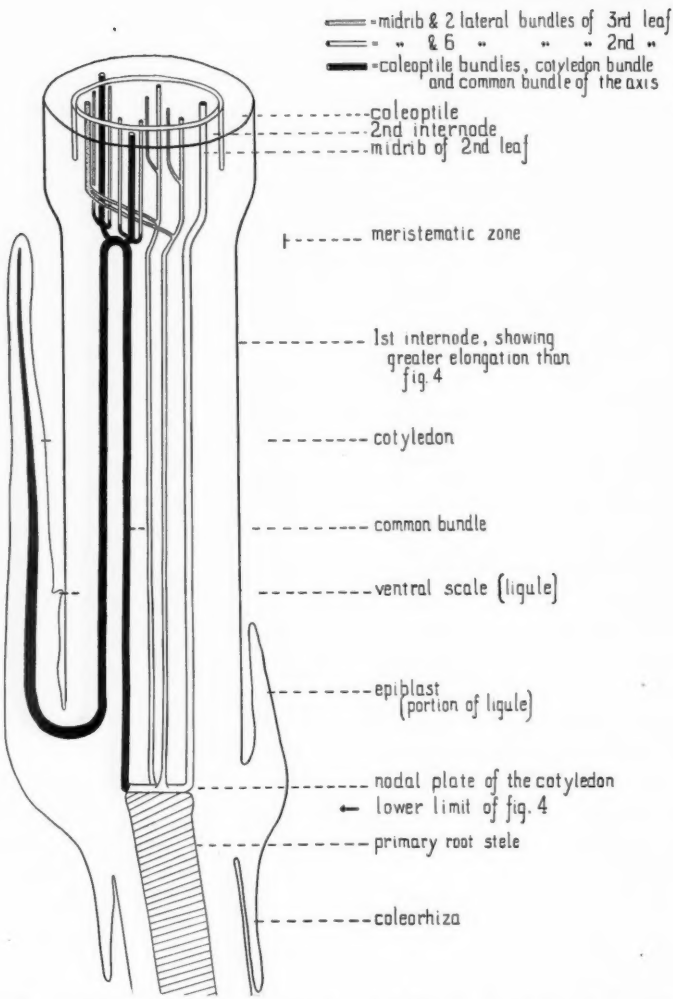

midrib & 2 lateral bundles of 3rd leaf
" " & 6 " " " 2nd "
coleoptile bundles, cotyledon bundle and common bundle of the axis
coleoptile
2nd internode
midrib of 2nd leaf
meristematic zone
1st internode, showing greater elongation than fig. 4
cotyledon
common bundle
ventral scale (ligule)
epiblast (portion of ligule)
nodal plate of the cotyledon
lower limit of fig. 4
primary root stele
coleorhiza

FIG. 3. *Avena sativa*, corresponding to fig. 1 for *Triticum*. First internode has undergone considerable elongation.

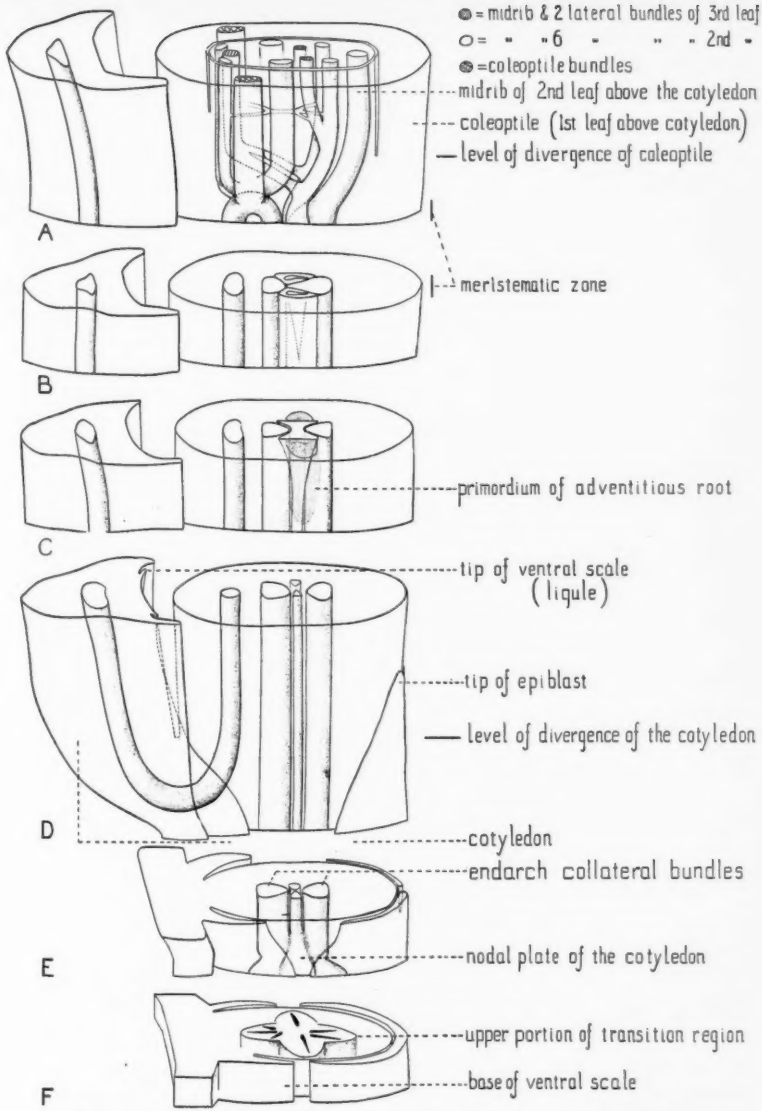


FIG. 4. *Avena sativa*, corresponding to fig. 2 for *Triticum*. First internode increases in length at its upper end, with new cells arising in meristematic zone indicated, and subsequently elongating.

lies near the epiblast, midway between the other two. In the seedling, two or three adventitious roots appear in the neighborhood of the meristematic zone (fig. 4 *B*). Less frequently a pair of roots arises in the middle of the internode or near its base. The seminal roots are usually hexarch. Two adventitious roots later emerge at the coleoptilar node. Of the seven xylem strands making up the polyarch stele of the primary root (fig. 4 *F*), three may be traced to the common bundle, two to the midrib of the second leaf, and one to each of the small interlying strands. The lateral strands (fig. 4 *F*) connect certain lateral bundles of the second, and the midrib and two lateral bundles of the third leaf with the primary root.

Discussion

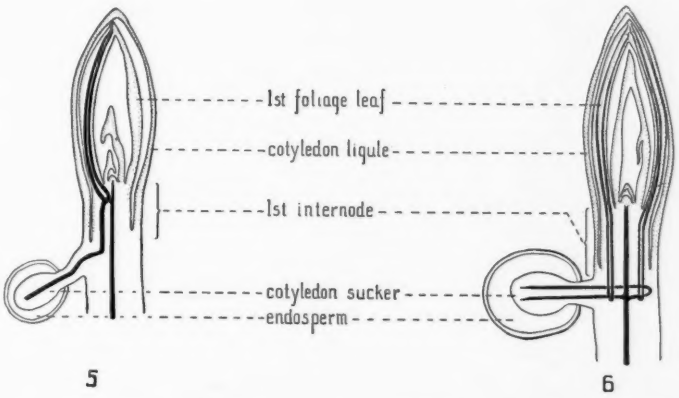
The axis between the transition plate and the coleoptilar node is the first internode of seedling grasses. Whether short or long it has an intermediate root-stem structure such as occurs in the first internode of diverse types of seedlings. Its lateral bundles (from the second and third leaves) are neither exarch nor endarch, but the two main bundles are normal endarch collateral bundles (figs. 2, 4).

That we are dealing with an ordinary internode and not a peculiar structure ("mesocotyl," "hypocotyl," "elongated node," etc.) is clear. The cotyledonary bundle branches to supply the coleoptilar traces at the point where it connects with the stele, and then extends downward to the transition plate. The term *cotyledon trace* is reserved for the bundle only to the point where it enters the stele; thereafter it is regarded as a common bundle. The anomaly of the coleoptile drawing its vascular supply directly and entirely from another leaf, the cotyledon, is thereby disposed of. A similar nomenclature would naturally apply to other monocotyledonous seedlings with an identical anatomy in the first internode (figs. 5, 6). That the bundle follows an uninterrupted course from the cotyledon to the transition plate is scarcely an argument against this nomenclature. Similar situations frequently exist at higher levels of the stems of numerous plants.

The normal leaf-axis relationship is obscured in monocotyledonous seedlings by the dominance of the cotyledon. In ontogeny the cotyledonary bundle (or bundles) differentiates while the stem is

still comparatively embryonic. In the ontogeny of higher leaves the main provascular strands similarly differentiate before connection with stem bundles is made.

McCALL (7) has presented detailed observations on the wheat seedling. He interprets that part of the axis between the cotyledonary and coleoptilar nodes, the first internode of AVERY (2) and BOYD (4), as two internodes. The first he places between the attachment of the epiblast and the cotyledon, the second between the



FIGS. 5, 6.—Fig. 5, *Billbergia zebrina:* diagram of two-weeks-old seedling. In its main plan the first internode is comparable with that of *Avena*. Fig. 6, *Alpinia calcarata:* diagram of week-old seedling, illustrating anatomy of first internode, which in its main features is comparable with that of *Triticum*, even though elongating somewhat.

levels of divergence of the cotyledon and the coleoptile; the epiblast is then the "first non-functional leaf," the scutellum the second leaf, and the coleoptile the third.

The vascular plate (fig. 2 *D*) formed by the moving inward of certain traces of the second and third leaves is interpreted by McCALL as the second node. He bases his interpretation on three tenets:

a. "*Vascular connections extend entirely across the central cylinder except for a small pith area.*" The occurrence of a vascular plexus does not necessarily imply the existence of a separate node. It is a region of anastomosing lateral traces of the second and third leaves.

An equally plausible interpretation is that it is the upper zone of a transition which seldom requires more than 0.5 mm.

b. "*The scutellar trace diverges from the axis below this plate.*" The question may well be raised as to what constitutes a node. Internal criteria alone are inadequate. According to an early definition (**6**), a node is "that part of a stem which has a leaf or a whorl of leaves." This interpretation is still in accepted usage. The cotyledonary attachment is more closely associated with the cotyledonary plate than it is with McCALL's plate, and externally its level of attachment extends appreciably above and below it. The plexus may be interpreted as a second node only if the scutellum can definitely be proved to be a second cotyledon; that is, that the epiblast is the first cotyledon. Evidence to support the contention that the epiblast is the first cotyledon would result in classifying the grasses possessing an epiblast with the dicotyledons. Such evidence is lacking. Accordingly, the divergence of the scutellar trace from below McCALL's "plate" does not, in our judgment, provide grounds for his interpretation.

c. "*Adventitious roots are associated with it as in any typical node.*" The presence of adventitious roots does not define a node. Nodal rooting is undoubtedly secondary. A nodal plexus may exist in rootless nodes and adventitious roots may arise in an internode. Comment has been made on the variability in the origin of the second pair of adventitious roots in *Triticum*. It is even more striking in *Avena*.

Certain difficulties arise from McCALL's application of his scheme to *Avena*. The horizontal trace which he labels "second node, scutellum trace divergence" presumably corresponds to the anastomoses of the midrib of the third leaf with two bundles, each comprising lateral traces of the second and third leaves (fig. 4 *A*). It is above the level at which the cotyledonary bundle leaves the stele, and has no direct connection with it. The region in *Avena* most closely approximating the structure of McCALL's second node in *Triticum* is to be found immediately below the meristematic zone in a region of active differentiation (fig. 4 *B*).

Use of the term hypocotyl (**7**) for the first internode is puzzling.

According to McCALL's own interpretation of the *Avena* seedling, he makes hypocotyl and first internode synonymous, and places the two structures which he regards as cotyledons (epiblast and scutellum) at the base of the hypocotyl.

From a study of *Sorghum vulgare*, REZNIK (9) notes that the axis between the cotyledonary and coleoptilar nodes shows a resemblance to the hypocotyl rather than to the higher part of the axis, and concludes that the region under discussion has not the structure of an internode but is a special structure, the nodal region of the scutellum. Since hypocotyledonary structure is so variable in different plants, and at different levels in the same plant, comparisons involving it are impossible. His main reasons for regarding the coleoptile as a dependency of the scutellum are those of previous investigators and have already been discussed (2, 3).

It is considered by ARBER (1) that the scutellum is equivalent to the blade of the foliage leaf, the coleoptile to its ligule, and the intervening axis designated "mesocotyl" to the cotyledonary node elongated by intercalary growth in its upper region. From our study it appears that the coleoptile bears no closer relationship to the seedling axis or to the scutellum than do the later leaves. A theory which makes the coleoptile part of the cotyledon is of questionable value in view of the occurrence (4) of similar vascular connections in the first internode of seedlings of *Billbergia zebrina* and at least four genera of Zingiberaceae. The first leaf in these seedlings would never be considered part of the normal complete cotyledon which they possess, nor would their first internode be regarded as an anatomical freak. ARBER further considers the epiblast and coleorhiza as non-foliar excrescences similar to the "rachilla flap" of certain Gramineae. In those instances with which we are familiar the epiblast and ventral scale consistently occur together and the key to their identity may be found in a study of the seedlings of the Bromeliaceae and Zingiberaceae cited, where the cotyledonary ligule is clearly the equivalent of the ventral scale and epiblast of the grass seedling.

It is difficult to understand the reluctance of students of grass seedling morphology to place the Gramineae in a more natural re-

lationship with the phylum and unfortunate that in this group emphasis frequently has been placed on anatomical differences rather than on likenesses.

Summary

1. A detailed study of the anatomy of the seedling axes of *Triticum vulgare* and *Avena sativa* provides evidence for the following interpretations:

a. The coleoptile is the first leaf above the single cotyledon; its divergence from the axis marks the coleoptilar node.

b. The first internode, whether short (*Triticum*) or long (*Avena*), extends from the cotyledonary to the coleoptilar node. It has intermediate root-stem structure with two main endarch collateral bundles and numerous more or less transitional strands. The old term mesocotyl implies that the first internode is part of the cotyledon; this is not the case, and the term should be dropped from the literature.

c. The hypocotyl which lies between the cotyledonary plate and the upper limit of primary root structure is so short as to be practically negligible.

2. The vascular relationships of seedling organs are discussed in some detail. The evidence from this study does not support recent theories which would make the epiblast-bearing grasses dicotyledonous or the seedling structure of Gramineae an anomaly among monocotyledons.

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FORMS OF CRATAEGUS PRUINOSA

H. W. RICKETT

(WITH EIGHT FIGURES)

In 1908 and 1911 SARGENT[1] listed 125 species of *Crataegus* from Missouri, most of them described and named by himself. Ten more were added in subsequent years.[2] The collections upon which his species were founded came from 22 counties; six of these form a group in the southwestern corner of the state, seven more are clustered in the southeastern Ozark region, three others are scattered in the Ozarks, three are near St. Louis and one near Kansas City; only four species were described from north of the Missouri River. These species, many of which are known only from the type locality, are associated with such assiduous collectors as *E. J. Palmer*, *J. Kellogg*, and *B. F. Bush* (32 species, for instance, are recorded only from Jasper County, having been collected there mostly by *Palmer*); the absence of records from the remaining four-fifths of the state is due not to the absence of *Crataegus* but to a lack of sufficiently careful collections (as SARGENT himself pointed out[3]). Furthermore, there is little doubt that proper collections would reveal a like profusion of species in many remaining parts of the state; it is evident that many of the *Crataegi* of Boone County, for instance, cannot be identified with any of SARGENT'S species.

The current aspect of the "*Crataegus* problem" almost justifies the prediction of ADANSON about the future of species,[4] and the attitude even of many taxonomists toward it is one of hopelessness. The description of additional forms would seem to be merely reckless. If, however, we are to understand any local flora, it is necessary that the distinguishable entities in this genus be collected and described and some provision made for a rational and usable nomenclature. For

[1] Rep. Mo. Bot. Gard. **19**:35–126. 1908; **22**:67–83. 1911.

[2] Trees and shrubs **2**:236–245. 1913; Jour. Arn. Arb. **1**:253. 1920; *ibid.* **3**:2–10, 82. 1922; *ibid.* **6**:2–4. 1925.

[3] Rep. Mo. Bot. Gard. **22**:67. 1911.

[4] Fam. Plantes; cited by BRITTON in Amer. Nat. **42**:226. 1908.

several years it has been one of my cares to attempt to learn something of the *Crataegi* of the vicinity of Columbia, Missouri. The present paper is limited to the few Pruinosae of this region, but treats these in a way which perhaps may contribute to the elucidation of the general problem.

It was long ago pointed out by such students as BRITTON[5] and BEADLE[6] that for a proper understanding of the species of *Crataegus* it is necessary to observe the plants in the living condition and at various times in the year. In order to study also the more minute characters it is desirable to collect from the same trees during successive years. A number of plants near Columbia have been marked with numbers; 20 of these being Pruinosae. These trees (with, of course, many others which were not labeled) I have had under close observation during three years (1931–1933). During parts of these years when I was absent I have had the valued assistance of Dr. FRANCIS DROUET. In the spring of 1934 collections and observations were made by Miss ROSE KENTNER. It is a pleasure to acknowledge my indebtedness to these helpers.

In *Crataegus*, as in other polymorphic genera, the few broadly conceived species of earlier workers have been replaced by a multitude of more homogeneous groups, often distinguishable only by minute characters; of such a nature are, for instance, the species described by SARGENT. Such groups have been termed elementary species or microspecies. The chief problem in the study of *Crataegus* is the taxonomic treatment of these groups; without prejudicing the conclusion finally reached, I shall refer to them rather loosely during the ensuing discussion as forms or types.

Three types of Pruinosae may be distinguished in the neighborhood of Columbia. They differ in a number of characters, such as color of anthers, number of stamens and of styles, margination of sepals, pubescence of leaves and of inflorescence, length of spines. The characters in themselves are unimportant and often difficult to detect, but the correlation between them is striking and interesting.

The most obvious differences are in the color of the anthers, these being red, rose-pink, or cream-white. The number of stamens in all

[5] Bot. Gaz. **22**:222. 1896.

[6] Bull. Torr. Bot. Club **37**:255. 1910.

plants seen is about 20; in view of the variations in number even among the flowers of one plant, this statement would suffice for ordinary systematic purposes. When, however, actual counts are treated statistically, significant differences appear between plants characterized by anthers of different colors. For this purpose, the stamens were counted in ten flowers of each plant, the mean and standard deviation being calculated for each plant so studied. The results are presented in table I. It will be noted that with three exceptions (numbers 2, 5, 17) plants alike in color of anthers are remarkably uniform in the mean number of stamens per flower, and these numbers are significantly different in two of the groups. Of the exceptions, two are exceptional only in one year; this makes it probable that the statistical methods used were not entirely adequate to deal with such a fluctuating character, and that the other exception may be similarly explained, particularly since this plant belongs to the group with pink anthers, in which the number of stamens seems to be regularly more variable. The number of styles was studied in the same way (the same flowers furnishing both series of figures); exactly the same statements may be made about the results.

The length of spines is an extremely variable and unsatisfactory character; but, as may be seen from the table, random measurements (made on herbarium specimens) of the spines mostly of young flowering or fruiting shoots show an obvious difference between two of the groups. This confirms the impression gained from field observation.

The sepals of all the plants are more or less glandular on the margins. Those of plants which bear white anthers, however, are very sparingly so, many of them being entire save for a few delicate cilia. Sepals of flowers which contain pink anthers are regularly provided with long-stalked glands on their middle portions. In spite of the apparent variability of this character, so close is the correlation between the nature of the margin of the sepals and the color of the anthers that it is possible to predict with confidence the color of the anthers in unopened flowers by an examination of a few buds; almost never does one make a mistake. The differences are illustrated in figures 1–8.

TABLE I

Plant no.	Mean number of stamens per flower	Mean number of styles per flower	Length of spines (mm.)
	Plants with white anthers		
2*	17.7 ±0.40	4.3 ±0.17	38.5 ±0.29
	19.6 ±0.27	4.9 ±0.06	
4	20.0 ±0.09	4.7 ±0.10	40.5 ±0.24
	19.3 ±0.26	4.5 ±0.10	
7	19.8 ±0.16	4.0 ±0.13	42.2 ±0.25
	19.3 ±0.25	4.4 ±0.10	
9			44.7 ±0.25
12			40.3 ±0.31
17	17.6 ±0.37	4.0 ±0.16	
	19.7 ±0.21	4.4 ±0.17	
18	19.4 ±0.19	4.2 ±0.13	38.1 ±0.20
Unlabeled plants	18.6 ±0.27	3.6 ±0.10	
	19.2 ±0.16	4.3 ±0.13	
	19.8 ±0.15	4.5 ±0.11	
	19.3 ±0.28	4.2 ±0.15	
	19.8 ±0.31	4.3 ±0.13	
	19.5 ±0.19	4.6 ±0.10	
	19.8 ±0.16	4.5 ±0.14	
	19.9 ±0.06	4.3 ±0.17	
	19.4 ±0.25	4.7 ±0.10	
	19.5 ±0.15	4.4 ±0.11	
	Plants with pink anthers		
3	17.4 ±0.35	3.4 ±0.10	25.7 ±0.22
5	19.2 ±0.31	3.8 ±0.16	26.7 ±0.21
	20.0 ±0.16	4.2 ±0.13	
15	18.6 ±0.27	3.7 ±0.18	
	17.4 ±0.33	3.0 ±0.19	
26			23.5 ±0.12
27			25.7 ±0.11
32			36.7 ±0.22
61	18.6 ±0.28	2.9 ±0.11	
Unlabeled plants	18.9 ±0.29	3.3 ±0.16	
	17.9 ±0.36	3.4 ±0.14	
	17.0 ±0.33	3.5 ±0.17	
	17.2 ±0.40	3.2 ±0.19	
	18.3 ±0.40	3.5 ±0.14	
	18.9 ±0.22	3.8 ±0.16	
	17.6 ±0.24	3.7 ±0.14	
	18.1 ±0.22	3.2 ±0.13	
	18.8 ±0.33	3.4 ±0.11	
	18.9 ±0.20	3.4 ±0.14	

* Two entries for one plant represent counts made in successive years.

TABLE I—*Continued*

PLANT NO.	MEAN NUMBER OF STAMENS PER FLOWER	MEAN NUMBER OF STYLES PER FLOWER	LENGTH OF SPINES (MM.)
	Plants with red anthers		
14	19.8 ±0.13	4.2 ±0.18	37.3 ±0.02
Unlabeled plants	19.5 ±0.14	4.2 ±0.18	
	19.7 ±0.13	4.3 ±0.10	
	19.8 ±0.08	4.2 ±0.13	
	19.5 ±0.17	3.9 ±0.18	

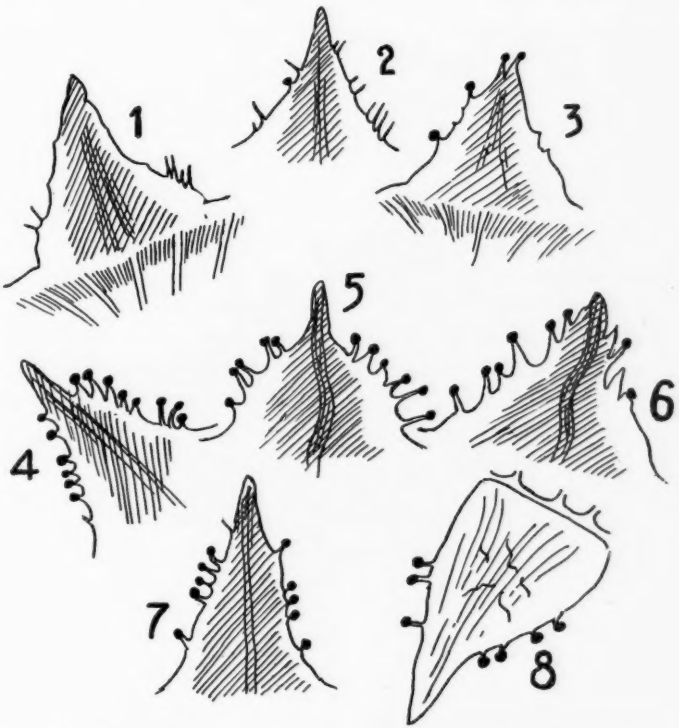
Almost all the plants are nearly or entirely glabrous. In this respect also, however, close examination reveals constant differences. The plants with red anthers are distinguishable from those with pink anthers by the presence on the upper surfaces of their leaves of a thin glistening pubescence; in maturity they may be slightly scabrid. The inflorescence also is sparingly pubescent. The plants with pink anthers are entirely glabrous from the first. Those with white anthers are sometimes glabrous; but frequently at least some of the young leaves bear on their upper surfaces a few short glistening hairs in the areas between the principal veins.

The types may be distinguished also by the size and number of the nutlets, and by other characters of fruits. I have not as yet satisfactory evidence of the uniformity of these characters.

The constancy of the preceding characters during the period of observation has been remarkable. The plants grow in several widely separated situations, mostly rocky, well drained hillsides (where they are mingled with other species of *Crataegus*). The forms characterized by pink and by white anthers grow together in all of the localities where the Pruinosae are abundant, and specimens of either type collected from different localities resemble each other as closely as do those from the same spot. The more pubescent form with red anthers has been found in only one place, where it is associated with the two others and with other species. Particular attention was given to trees growing in close association, in order to detect any signs of intergradation between the different types; the results were entirely

negative, plants so close that their branches were interlaced, and blooming at the same time, being easily separable into the groups just described.

It is not, of course, proposed that systematists should be prepared



FIGS. 1–8.—Sepals of Pruinosae: figs. 1, 2, 3, plants with white anthers; figs. 4, 5, 6, plants with pink anthers; figs. 7, 8, plants with red anthers. ×5.

to make statistical calculations of the numbers of stamens or of styles before being able to determine the name of a species, variety, or form; nor that the characters which have been used in this study are necessarily of value in other sections of the genus.[7] But the existence

[7] PALMER writes that the glands of the sepals, for instance, are so variable in some species as to be of small taxonomic value.

of such types and the possibility of such distinctions as those described in this paper warrant certain conclusions, which may be of general value.

The cultivation of *Crataegus* at the Arnold Arboretum has demonstrated the remarkable constancy of species when grown from seed.[8] Many of the species, nevertheless, including *C. pruinosa*, are thought to be of hybrid origin. This is based on their polyploid chromosome numbers and on the large proportion of sterile pollen grains associated with irregularities in meiosis.[9] Polyploidy is often taken as indicating hybridity, although of course it does not necessarily do so.[10] In any case, the existence of constant lines within a species marked by both polyploidy and pollen sterility makes it probable that the reproduction is apomictic.[11] The close correlation of a number of small characters in each of the three types described in this paper, and the entire absence of any individuals which show the same characters in different combinations, also indicate that each type propagates itself without interbreeding and probably apomictically. Experiments by SAX, reported by PALMER (*l.c.*: 357), have shown that castrated flowers of a number of species, including *C. pruinosa*, set fruit at least sometimes.

The connection of polyploidy, hybridity, and polymorphism with abnormalities in reproduction is well known.[12] The method of reproduction cannot, of course, be inferred from a knowledge of the chro-

[8] PALMER in Jour. Arn. Arb. **13**:350. 1932; BROWN in Bull. Torr. Bot. Club **37**: 254–255. 1910.

[9] LONGLEY (Amer. Jour. Bot. **11**:300. 1924) reports *C. pruinosa* a triploid with "24 diploid chromosomes." He calls species with 16 bivalents diploid and those with "32 gametophytic chromosomes" tetraploid. MOFFETT (Proc. Roy. Soc. Lond. B **108**:438; fig. 50. 1931) lists the same species as having a reduced number of 34, and calls it tetraploid (not diploid, as PALMER says, *l.c.* :357), the basic number of the genus being, as in other Pomoideae, 17. It is possible that different forms of this and other species are characterized by different chromosome numbers (see also SAX in Jour. Arn. Arb. **12**:7. 1931). For pollen sterility in *Crataegus* see LONGLEY, *l.c.*: 313. 1924; STANDISH in Jour. Hered. **7**:266–278. 1916; SAX, *l.c.*, figs. 1, 6, 7, 8.

[10] See, for instance, JEFFREY, LONGLEY, and PENLAND in Sci. **55**:517–518. 1922; and compare the views of SAX (Jour. Arn. Arb. **13**:363–367. 1932) and MOFFETT (*l.c.*: 423–446) on the origin of the chromosome number 17 in *Malus*.

[11] *Cf.* SAX in Jour. Arn. Arb. **12**:18. 1931.

[12] BLACKBURN and HARRISON in Ann. Bot. **35**:184. 1921; WOODWORTH in Jour. Arn. Arb. **12**:214. 1931.

mosomal abnormality. In *Malus theifera* according to SAX,[13] and in species of *Rosa* according to HURST,[14] the course of meiosis is entirely different in megaspore and microspore mother cells; most of our knowledge of polyploidy in *Crataegus* and other genera is based almost entirely upon a study of pollen and is strikingly silent on events in the ovule. Castration experiments also may give little information upon the origin of the embryo. In *Zygopetalum*, according to SUESSENGUTH,[15] although pollination is necessary to the formation of the embryo, the pollen tubes do not penetrate the ovules and the embryos arise apomictically. In *Allium odorum*, as described by MODILEWSKI,[16] parthenogenetic embryos arise from diploid cells of the embryo sac only if pollination has occurred and one of the male nuclei has united with the polar nuclei. Similar phenomena of "pseudogamy" are indicated by experiments with *Rubus*[17] and *Potentilla*.[18] The cross pollinations reported by BROWN (*l.c.*, 259) give consequently little information on the actual source of the embryos of *Crataegus*. They may perhaps be formed in a number of different ways even in one species or variety, and these methods may include both apomixis and occasional normal syngamy, as in *Allium odorum* (MODILEWSKI, *l.c.*).

The constancy of the different types might result, as PALMER (*l.c.*: 356) suggests, from physiological differences which prevent interbreeding; effective cross pollination might fail through slight differences in the dates of shedding of pollen and of receptivity of stigma. It is true that the white-anthered type here described blooms slightly earlier than the others; but the flowers of one plant do not open all at once, and the flowering periods of the different types overlap. Even though such conditions may reduce the likelihood of hybridization in otherwise normally amphimictic races, the existence of bad pollen and of polyploidy, with such a high degree of constancy, makes apomixis almost certainly the usual method of reproduction.

[13] Jour. Arn. Arb. **13**:365. 1932.

[14] Zeit. Ind. Abst. Vererb. suppl. **2**:891 ff. 1928.

[15] Ber. Deutsch. Bot. Ges. **41**:16–23. 1923.

[16] Ber. Deutsch. Bot. Ges. **48**:285–294. 1930.

[17] LIDFORSS in Zeit. Ind. Abst. Vererb. **12**:1–13. 1914.

[18] MÜNTZING in Hered. **11**:267–283. 1928.

The existence of apomictic lines is not, of course, incompatible with their origin through hybridization. Such a situation is known or suspected in other polymorphic genera, such as *Hieracium*,[19] *Rosa*,[20] *Rubus*,[21] and *Betula* (WOODWORTH, *l.c.*).

When we turn from the description of races to the problem of naming them, the troubles of the taxonomist begin. The application of the traditional species concept to the great polymorphic genera seems to have become very difficult if not impossible. One source of trouble is that the concept of species is, as has been often pointed out,[22] descended from pre-evolutionary times. The Linnean concept of a species (as has been pointed out by BLAKE,[23] by RAUNKIAER,[24] and by CHASE[25]) was not a broader concept than ours; it was not intended to embrace an aggregate of subordinate units, but was a homogeneous group maintaining itself through reproduction. (The varieties of LINNÉ and his contemporaries were ascribed to environmental differences, or to hybridization.[26]) With the development of modern technique the distinguishable units have become smaller and smaller,—the so-called microspecies; and with the recognition that species may be still in the making interest has become centered upon groups of approximately uniform genic constitution,—the biotypes (genotypes) of JOHANNSEN or the isoreagents of RAUNKIAER (*l.c.*: 230, 236). Such are the races of *Crataegus* described in this paper and probably most of the species which have been described in this genus. Meanwhile the idea of the species, with its binomial burden, has striven desperately to adapt itself to the new outlook. On the one hand, species names have threatened to become so multitudinous as to be useless, on the other so broad as to be meaningless; in no case can they fulfill the original definition of a species.

The limits and homogeneity of species have varied with the assiduity and interests of the botanists concerned, so that there is some

[19] OSTENFELD in Zeit. Ind. Abst. Vererb. **3**:241–285. 1910.

[20] HURST, BLACKBURN, and HARRISON, *l.c.*

[21] LONGLEY in Amer. Jour. Bot. **11**:249–282. 1924.

[22] BAILEY in Bot. Gaz. **22**:454. 1896 and Proc. Int. Cong. Plant Sci. **2**:1428. 1929; HARPER in Amer. Jour. Bot. **10**:229–233. 1923.

[23] Rhod. **20**:22. 1918.

[24] Zeit. Ind. Abst. Vererb. **19**:226. 1918.

[25] Amer. Jour. Bot. **8**:48. 1921.

[26] Phil. Bot. 100. 1751; Gen. Plant. 1764.

justification for the common point of view expressed by BAILEY,[27] GRAY,[28] SHULL,[29] and many others, that the concept of species is mainly a matter of convenience. On the other hand the system of LINNÉ would not have been possible had not his species corresponded to something in nature. Although the Linnean concept is no longer valid, many of the groups of plants which LINNÉ named as species are still recognized as such. In short, the microspecies seem to group themselves into larger assemblages, just as the latter group themselves into genera. As MÜNTZING, TEDIN, and TURESSON state: "We are free to discuss which of these units should be called species, since we do not know 'Nature's own opinion on this matter.'"[30]

It is becoming generally recognized that to treat as species the vast number of recognizable types found in such genera as *Crataegus* is neither practicable nor desirable.[31] Strangely enough, to consider such types species savors strongly, as COWLES pointed out,[32] of a return to a creationist point of view—the belief in fixed units. Actually the Linnean concept is no more applicable to them than to anything else in nature, for even these smaller groups are not necessarily true breeding nor constant, and a nomenclature which recognized them explicitly as such would become more and more chaotic with the passage of time. In spite of the success of genetics, the methods of that science, as BRITTON[33] and RAUNKIAER (*l.c.*, 233–235) have shown, cannot replace the methods of taxonomy in describing the units of classification; species and their subdivisions must continue to be founded on the similarities observed in nature, not on pedigrees nor chromosomes. If we are to have something to call a species, it must be something rather constant.

Among the many recent attempts to devise a system for the naming of subspecific units, the work of TURESSON is well known.[34] He

[27] Bot. Gaz. **22**:457. 1896.

[28] Amer. Jour. Bot. **10**:222. 1923. [29] Darwiniana 35. 1889.

[30] Hered. **15**:8. 1931; *cf.* remarks of DE WILDEMAN in Proc. Int. Cong. Plant Sci. **2**:1413–1421; and of WIEGAND, *ibid.*: 1575. 1926.

[31] The classic defense of "splitting" is RYDBERG'S paper in Proc. Int. Cong. Plant Sci. **2**:1539–1551. 1926.

[32] Amer. Nat. **42**:268. 1908. [33] Amer. Nat. **42**:238. 1908.

[34] Hered. **3**:100–113, 211–350. 1922; **6**:147–236. 1925.

has shown that differences among the plants of a single species which have usually been ascribed to environmental factors often represent "ecotypes" of different hereditary constitutions. The ecotypes correspond roughly to the varieties of conventional taxonomy, and their naming presents no serious difficulty (whether old names be preserved or TURESSON'S suggestions be followed). These groups are not, however, comparable to the microspecies of the large polymorphic genera; each may be a group of different biotypes, often capable of free interbreeding, the entire complex being held constant largely by the selective action of the environment. The taxonomic problem is more acute in such genera as *Viola* and *Rosa*, in which a single broadly conceived species may consist of an almost indefinite number of interbreeding races, spread over a wide territory, and all entitled to some taxonomic recognition. Any maker of names may well pause before the more than five million such forms mentioned by CLAUSEN[35] in the collective species *Viola tricolor*. There is much to be said for a system of numbers for the subordinate units of such species, as was long ago suggested by BRITTON.[36] CLAUSEN used symbols for the forms of *Viola* which he studied; more recently ERLANSON[37] has treated various forms of *Rosa* by means of numbers and letters. Certain species (called agamospecies by TURESSON[38]) differ from those just mentioned in that their component races are apomictic, and therefore constant. An example described by TURESSON is *Festuca ovina*, which in certain regions consists of "viviparous" races.[39] A similar situation exists in certain sections of *Hieracium* (OSTENFELD, *l.c.*). *Crataegus pruinosa* and perhaps other species (*sensu lato*) of *Crataegus* seem to be of this type.

While the number of races of *Crataegus pruinosa* may be very large (particularly since, as was pointed out at the beginning of this paper, there is no reason to think that even in this one state all have been collected and described), the situation is perhaps not so disheartening as in some species of *Viola* and *Rosa;* because of the method of reproduction, and for other reasons, single races are not widespread and the number found in any one locality is not very large. I ven-

[35] Bot. Tids. **37**:392. 1922.

[36] Amer. Nat. **42**:241–242. 1908.

[37] Bot. Gaz. **96**:197–259. 1934.

[38] Hered. **12**:323–334. 1929.

[39] Hered. **8**:160–206. 1926.

ture, therefore, to append formal names and descriptions of the types described in this paper. They will be useful only to students of a limited portion of Missouri, and among them only those willing to devote careful observation to the plants; however, if a similar disposition is made of the forms peculiar to other localities, the collective species as a whole will be the better known.

A difficulty with present nomenclature is the lack of definition of the subspecific categories which are prescribed. In describing the races of *Crataegus pruinosa* as forms, I am following what seems to be general practice;[40] such forms also correspond to the *forma apomicta* of TURESSON.[41] Some botanists, however, such as CLAUSEN (*l.c.* 396), designate as forms merely environmental modifications. It is probable that methods of naming should differ in different genera. Whatever be the designation of subordinate races, it should be recognized that none of them is to be considered the "true species" from which the others diverge (except, of course, for nomenclatural typification). If species are to be broadly conceived as composed of many distinguishable groups, some sort of trinomial system must come into general use; agreement on the methods to be used may well be a sign that taxonomic nomenclature has definitely outgrown its heritage of pre-evolutionary thought.[42]

A survey of all the Pruinosae may show that it is possible to distinguish among them several large species, in which the multitude of forms may be classified. Failing clear morphological distinction, it may perhaps be feasible to create geographical species, purely for convenience in listing the forms, which seem to be largely local. Whatever method of treatment finally emerges, it should give proper recognition to the hundreds of microspecies described by SARGENT and others; they cannot be retained as species, but they represent nevertheless real groups.[43]

[40] See WIEGAND, *l.c.*: 1575. *Cf.* COCKERELL'S note in Torr. **34**:42. 1934.

[41] Hered. **8**:160–206. 1926.

[42] The Linnean concept is still represented by such recommendations as those of LAUJOUW in Fedde Repert. Sp. Nov. Beih. **66**:200. 1932. He argues that varieties are divergents from a true specific type.

[43] Through the kindness of the staff of the Herbarium of the Missouri Botanical Garden I have been able to examine collections from the type localities, often from the

It may be impossible to identify one of the Pruinosae as *forma genuina*, since no type specimen of *C. pruinosa* is known, and the original description [44] is naturally not sufficiently detailed to permit of distinction from the multitude of known forms. KOCH, who transferred the species to *Crataegus*,[45] left specimens now in the Herbarium of the Botanic Garden at Berlin.[46] One from ENGELMANN, collected near St. Louis, labeled in KOCH's hand, has about 20 stamens, from 4 to 5 styles, strongly glandular sepals, is somewhat pubescent, and has cordate leaves. Another sheet is labeled (also by KOCH) "*β icosandra* (*chlorocarpa*)." It has sepals nearly entire, leaves more tapering at the base. Neither of these fits WENDLAND'S description in the characters which distinguish the forms of the species. They serve merely to show that the specimens grown in Europe from which the species was named already included several forms.

Specimens of the following new forms are deposited in the Herbarium of the University of Missouri.

CRATAEGUS PRUINOSA (Wendl.) Koch **ciliata** f. nov.

Frutex gracilis; folia basi cuneata, fere glabra interdum supra paucis setis brevibus praedita; ramuli purpurei, glauci, post castanei tandemque cinerei; spinae similes, circiter 40 mm. longae, rectae vel leviter curvae; inflorescentia glabra, 3–5 floribus; sepala triangulato-acuminata, circa 4 mm. longa, marginibus scariosis saepe integribus vel leviter glandulosis et paucis ciliis exiguis praeditis; antherae eburnae; stamina circiter 19 (15–22); styli circiter 4.5 (3–5); fructus rubri, pruinosi, depresso-globosi, 10–15 mm. lati; nuculae 4–5, angulatae vel late costatae, 6 mm. longae.

Rickett, May 3, 1931 (Herb. Univ. Missouri 8888); *Drouet*, Oct. 4, 1931 (Herb. Univ. Missouri 9535).

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type trees, of most of SARGENT'S Missouri Pruinosae. SARGENT'S descriptions, also, are sufficiently detailed so that one can easily distinguish them from each other and from the forms described in this paper.

[44] *Mespilus pruinosa* Wendl. in Flora **6**:699–701. 1823.

[45] Hort. Dendr.: 168. 1853.

[46] I am indebted to DIELS for the privilege of examining these specimens.

CRATAEGUS PRUINOSA (Wendl.) Koch **glaberrima** f. nov.

Frutex gracilis; folia basi cuneata vel truncata, glabra; ramuli virides vel purpurei, glauci, post aurantiaci tandemque cinerei; spinae similes, circiter 30 mm. longae, leviter curvae; inflorescentia glabra, 3–6 floribus; sepala triangulato-acuminata, circa 4 mm. longa, marginibus scariosis glandulosis incisis; antherae roseae; stamina circiter 18 (14–22); styli circiter 3.5 (2–5); fructus rubri saepe viridi-maculati, pruinosi, depresso-globosi, 10–15 mm. lati; nuculae 3–5, saepe late canaliculatae, 7 mm. longae.

Rickett, May 3, 1931 (Herb. Univ. Missouri 8890); *Drouet*, Oct. 4, 1931 (Herb. Univ. Missouri 9558).

CRATAEGUS PRUINOSA (Wendl.) Koch **puberula** f. nov.

Frutex vel arbor compacta; folia basi truncata vel subcordata, supra parce pubescentia setis brevibus nitidis, subtus glabra; petioli pubescentes; ramuli virides vel purpurei, glauci, post fulvi tandemque cinerei; spinae purpureae, glaucae, post atro-rubrae nitentesque, tandem canae, circiter 35 mm. longae; inflorescentia parce pubescens, 3–5 floribus; sepala lanceolata, circa 5 mm. longa, parce glanduloso-serrata; antherae rubrae; stamina circiter 19.5 (18–21); styli circiter 4 (3–5); fructus virides vel rubescentes, pruinosi, depresso-globosi, 10–20 mm. lati; nuculae 4–5, canaliculatae, 6 mm. longae.

Rickett, May 5, 1931 (Herb. Univ. Missouri 8901); *Drouet*, Oct. 8, 1931 (Herb. Univ. Missouri 9544).

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PHOSPHORUS RELATIONS OF LEMON CUTTINGS GROWN IN SOLUTION CULTURES

A. R. C. HAAS

(WITH SIX FIGURES)

A study was made of the concentration of phosphate in the culture solution in relation to the growth and composition of lemon cuttings. Various concentrations of phosphate in the culture solution affect the absorption of other elements, and when an element becomes a limiting factor, the accumulation of high concentrations of other nutrient elements follows.

Rooted Lisbon lemon cuttings were grown in an unaerated culture solution lacking phosphate. This solution is that described by HAAS (5) modified by the substitution of potassium nitrate for potassium acid phosphate. Different concentrations of phosphate were added as potassium acid phosphate. The cultures were grown in $8\frac{1}{2}$ quart capacity Swedish enamelware pans. In some cases pans of 21 quart capacity were used for the low phosphate concentrations, but without affecting the growth.

It was desirable to sample the culture solutions from time to time in order to ascertain whether the phosphate concentration in the solution was being maintained. At first this was attempted as a guide for removal of the culture solution, but the facilities at hand did not allow its continuance.

In the preliminary experiments the solutions were renewed every other day. For a time the solutions of the duplicate set of cultures were renewed every day, with no resulting improvement. Sharp contrasts of growth within a relatively narrow range of phosphate concentration were not found, and consequently the cultures were grouped into somewhat wide ranges of phosphate concentration. It was desirable to maintain a given phosphate concentration in the solution continuously, yet this was possible in theory only, since the lemon cuttings require iron as well as phosphorus. When iron and phosphorus are both present at the same time in the culture solution,

varying amounts of both are precipitated. In practice, therefore, it was found best to maintain the phosphate for a time and then remove it; another method used was to allow the phosphate to remain while an abundant supply of iron was added.

The first set of cultures was conducted in duplicate, and the solution in one group contained a trace of aluminum while the solution in both groups contained ferric tartrate. The aluminum and iron were precipitated by the phosphate, which greatly altered the concentration of soluble phosphate. The concentrations of phosphate added to the culture solution were 0, 0.0125, 0.0250, 0.050, 0.075, 0.1, 0.2,

TABLE I

PHOSPHORUS AS A PERCENTAGE OF DRY MATTER OF LISBON LEMON CUTTINGS*

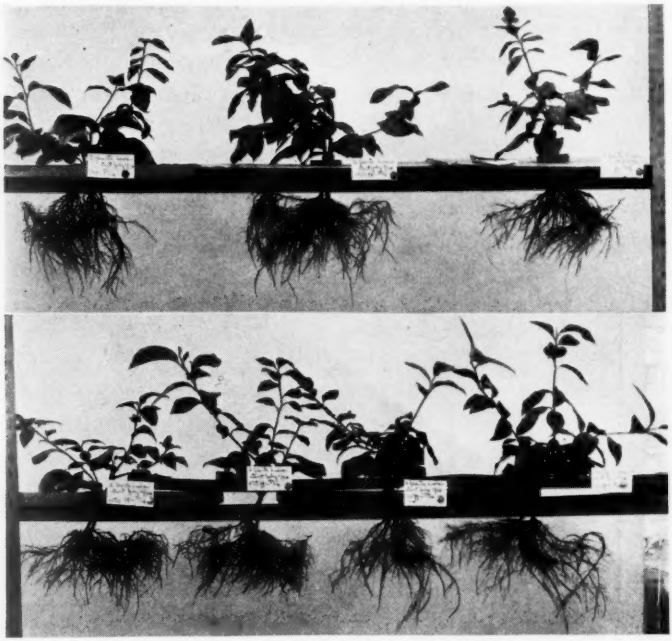
PO_4 IN CULTURE SOLUTION (P.P.M.)	ORIGINAL LEAVES	NEW LEAVES	ROOTS
0	0.0593	0.0556	0.0870
0.0125	0.0517	.....	.....
0.05	0.0508	0.0820	.....
0.10	0.0467	0.0674	.....
0.20	0.0545	0.0641	0.1192
105	0.0847	0.1879	.....

* Cuttings grown from January 26 to July 9, 1934, in solution cultures presumed to contain certain low concentrations of phosphate.

and 105 p.p.m. The percentage of phosphorus in the various portions of a number of the cuttings was determined at the conclusion of the experiment. The data are shown in table I.

The results give little information other than that at the highest phosphate concentration the original leaves of the cuttings contain a fairly high percentage of phosphorus. In such studies it is essential to keep iron out of the solution during the intervals in which the phosphate concentration is being maintained. Studies of phosphate nutrition involve, therefore, a consideration of the iron nutrition. The growth and composition of citrus cuttings will differ, not only according to the composition of the culture solution, even as regards certain traces of elements, but also according to the technique employed in using these solutions. Environmental variations add further to the complexity of the results.

Another set of Lisbon lemon cultures run in duplicate was begun on July 3 and concluded on December 19, 1934. The solutions were not aerated but were changed every few days and were given iron a few hours prior to such changes. In order to simplify the labor in-

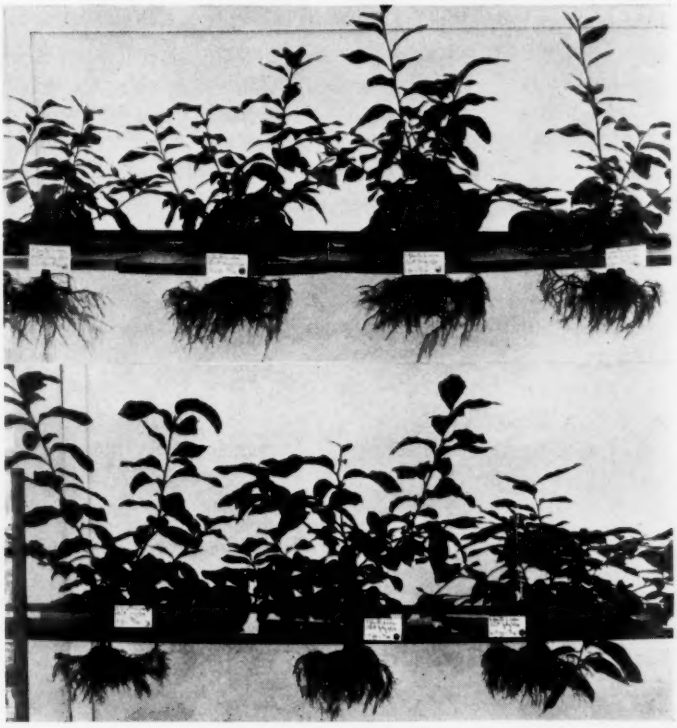


FIGS. 1, 2.—Lisbon lemon cuttings grown from July 3 to December 19, 1934, in unaerated solution cultures containing various concentrations of phosphate. Left to right: fig. 1, 0, 0.0125, and 0.025 p.p.m.; fig. 2, 0.050, 0.075, 0.1, and 0.2 p.p.m.

volved, no aluminum was used in these cultures. The following concentrations of phosphate as potassium acid phosphate were added to a culture solution lacking phosphate: 0, 0.0125, 0.025, 0.050, 0.075, 0.1, 0.2, 0.3, 0.5, 1.0, 2.0, 5.0, 7.5, 10.5, 15.75, 21.0, 26.25, 31.0, and 105 p.p.m. To secure a relative increase in the volume of solution at the lowest concentrations, the cultures each contained only two cuttings, while at higher concentrations the number was increased to three. The cuttings were photographed and then were grouped into

certain ranges of phosphate concentration. The growth obtained for each of these ranges is shown in figures 1–4.

Figures 1 and 2 illustrate the growth of cuttings in solution cultures containing from 0 to 0.2 p.p.m. phosphate. Only two cuttings



FIGS. 3, 4.—Same as figs. 1, 2. Left to right: fig. 3, 0.3, 0.5, 1, and 2 p.p.m.; fig. 4, 5, 7.5, and 10.5 p.p.m.; cuttings with the best root systems occurred in the cultures containing 1 to 2 p.p.m. phosphate.

were used in each of the $8\frac{1}{2}$ quart capacity pans. None of the plants could be said to be free of symptoms of phosphorus deficiency, regardless of how frequently it was possible to renew the solutions. No concentration of phosphate within this range proved superior to the others for the growth of the cuttings employed.

Figures 3 and 4 show the growth typical of duplicate cultures of Lisbon lemon cuttings produced in solutions containing from 0.3 to 10.5 p.p.m. phosphate. Three cuttings were used in each culture. The healthiest appearing root systems in conjunction with a top that just has sufficient phosphorus are seen to occur in figure 3 at roughly 1 to 2 p.p.m. Very slight symptoms of phosphorus deficiency occurred in the external appearance of young leaves from cuttings grown in a culture solution containing 1 p.p.m. phosphate but none at 2 p.p.m. At 5 p.p.m. and higher, the rootlets became more finely divided and less healthy, the cause for which will be pointed out later.

A photograph was made of the growth obtained when the duplicate cultures, each containing three plants, were grown in a solution containing from 15.75 to 105 p.p.m. of phosphate. The growth produced was not appreciably different from that shown in figures 3 and 4.

From experience the conclusion would be reached that at 1 p.p.m. phosphate there is slight danger of a phosphorus deficiency in the leaves, but above this concentration there is an adequate phosphorus supply for vegetative (non-fruiting) growth. Excellent top growth resulted at all concentrations above 5 p.p.m. but the roots suffered at the higher concentrations.

It will be noted by comparing the growths of the lemon cuttings in figures 1 to 4 that the longest roots were produced in the low range of phosphate concentration and the shortest roots in the highest range. This could hardly be due to the osmotic effect of the culture solution because the original solution contained approximately 1455 p.p.m. total solids, and the extreme variations did not exceed roughly 200 p.p.m. Darkening of the roots occurred in both of these ranges of phosphate concentration, but root breakdown occurred only in the highest range.

Above the range in which deficiency symptoms are seen, there is always the possibility of confusing excellent top growth at the moment with permanently healthy growth during which fruit production takes place. It could be said that excellent cuttings were grown at the 5.0, 7.5, and 10.5 p.p.m. phosphate concentrations; with other conditions of the solution favorable, these concentrations do not appear excessive.

The photographs illustrate the fact that there is no sharp division line as to what is the best phosphate concentration for growth, but rather that there is a range of concentrations that give equally good results. The best concentration range of phosphate for the growth of citrus holds only for that particular solution, degree of aeration, state of growth, and environmental condition. Variation in any one of these and other factors may cause a shift in the range of favorable concentration. In fact, growth frequently may be greatly benefited by a deficiency of phosphate, but the degree of such benefit has its injurious limits. Young citrus cuttings grow best in a culture solution lacking phosphate, but later in their growth they are greatly helped by the presence of considerable phosphate. Figures 1 to 4 show the gross symptoms of phosphorus deficiency; at close range they are still detectable at the 1 p.p.m. phosphate concentration.

Other investigators (11) have obtained a maximum growth of barley in culture solutions containing 1 p.p.m. phosphate; corn and beans at 0.5, and also at 0.25 p.p.m.; and sorghum and tomatoes at 0.5 p.p.m. It is conceivable that larger culture vessels and more frequent changes of solutions might have permitted better growth at the phosphate concentrations used with Lisbon lemon cuttings. However, no encouraging signs were seen in this direction when a reduced number of cuttings were used per culture and when more frequent changes of solution were made. PARKER and PIERRE (8) and also TIDMORE (11) have not as yet obtained good growth in cultures at concentrations comparable with those in the soil solutions of rather productive soils.

In the absence of available phosphates in the soil, but with an abundance of iron and aluminum, HOFFER and CARR (6) found corn roots susceptible to root rots. The nature of the plant appears to be important in phosphate nutrition. PFEIFFER, SIMMERMACHER, and SPANGENBERG (9) found that large applications of readily soluble phosphates were beneficial to oats but were injurious to buckwheat and lupine.

The role of phosphate in biological oxidations has been discussed by LYON (7) and by BARMORE and LUCK (1). It is possible that at high phosphate concentrations there is an increase in the respiration

of citrus roots, and under such conditions greater aeration may be required.

Reference to unhealthy roots accompanying high phosphate in unaerated culture solutions has already been made. Brief consideration of additional cultures makes it clear that aeration is of the greatest importance for citrus roots, especially when the phosphate concentration is high. The aeration of the roots by frequent changes of solution with the daily addition of aerated distilled water to make up the water losses from shallow culture pans is inadequate under these high phosphate conditions. Figure 5 shows lemon cuttings grown in a culture solution containing at all times approximately 105 p.p.m. phosphate (the iron being added just prior to changing the solution), while figure 6 shows lemon cuttings grown in a solution lacking phosphate to which was added 5–10 gm. of insoluble ferric phosphate. Both culture solutions were vigorously aerated and were contained in Swedish enamelware pans 18 inches in diameter at the top, 13 inches in diameter at the bottom, and 6 inches high.

In both cultures there was excellent top and healthy root growth. The use of abundant aeration prevented the roots of the cuttings grown with 105 p.p.m. phosphate or with insoluble ferric phosphate from becoming unhealthy up to this stage of growth. Because the cuttings require both iron and phosphate, the solubility of the insoluble ferric phosphate is thereby assumed to be accelerated. During hot weather under glasshouse conditions, the mature leaves of the ferric phosphate culture burned somewhat, which points to a possible inadequate phosphorus nutrition from this source under these particular environmental conditions. The top growth appears to be more extensive and the root system more finely divided when the phosphate supply is just barely adequate, as shown in figure 6. However, the actual fresh and dry weights of the leaves and twigs per cutting were somewhat less when the ferric phosphate was used.

These aerated cultures were grown for many months and their excellent growth makes it appear that phosphate nutrition in citrus may be intimately related to the processes of oxidation. These and other experiments suggest that the roots of citrus cuttings require either more oxygen or withstand less carbon dioxide than is ordinarily assumed, and that there may be a relation between the re-

FIGS. 5, 6.—Lemon cuttings grown in aerated culture solution: fig. 5, approximately 105 p.p.m. phosphate present at all times; fig. 6, about 10 gm. of insoluble ferric phosphate used as sole source of phosphate.

sponse to a given fertilizer element and the degree of root aeration. These are important problems for future investigation.

A grouping of the cuttings (figs. 1–4) at the time of harvest, according to the phosphate concentration, simplifies the consideration of the percentages of phosphorus and other constituents of the dry matter in the various portions of the cuttings. In table II the data show that the percentages of phosphorus increase with increasing phosphate concentrations in the culture solution.

TABLE II

PHOSPHORUS CONTENT IN RELATION TO THAT OF SUGAR IN VARIOUS PORTIONS OF CUTTINGS*

PO_4 IN CULTURE SOLUTION (P.P.M.)	PHOSPHORUS AS PERCENTAGE OF DRY MATTER				REDUCING SUGARS AS PERCENTAGE OF DRY MATTER			NON-REDUCING SUGARS (CHIEFLY SUCROSE) AS PERCENTAGE OF DRY MATTER		
	ORIGINAL LEAVES	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS
0	0.0775	.....	0.0563	0.1000	.....	0.27	0.84	.....	0.53	0.73
0.0125 to 0.075	.0850	0.0938	.0585	.1035	1.94	1.05	.74	1.46	2.34	1.50
0.1 to 0.5	.0863	.1063	.0663	.1250	1.83	0.90	.92	1.47	1.52	1.22
1 to 10.5	.0975	.1750	.1219	.1900	3.60	1.66	.78	2.12	2.71	2.38
15.75 to 31.5	.1225	.2188	.2041	.2750	2.97	1.50	.71	3.68	3.68	4.55
105	0.1300	0.2469	0.2375	0.4750	2.66	1.99	0.80	3.99	2.15	3.08

* Cuttings grown from July 3 to December 19, 1934, in solution cultures at different phosphate levels.

The culture solution employed contained 718 p.p.m. nitrate, which is large when compared with the phosphate concentrations used. It is reasonable to assume that the use of large volumes of a culture solution containing much less nitrate would permit better growth at the low concentrations of phosphate in which the cuttings showed symptoms of phosphorus deficiency. Knowledge of the actual phosphorus content of the soil solution is therefore particularly of value, and perhaps of value only, in relation to the concentration of other elements such as nitrate.

Table II also shows the percentages of reducing and non-reducing sugars in the dry matter of the cuttings. In this table the new mature

leaves of the cultures grown without phosphate were not analyzed but were frozen and the juice was extracted under 10 tons of pressure. The juice showed a pH value of 6.06 at 23° C. by the use of the quinhydrone electrode. Leaves of the cultures grown in solutions containing 105 p.p.m. phosphate treated in a similar manner at the same time showed a value of pH 6.52. Phosphate deficient leaves therefore have a greater acidity than have healthy leaves. In this respect they resemble mottled leaves which contain an excess of phosphorus.

The increase of acidity brought about by a deficiency of phosphorus confirms the results of ECKERSON (4) on tomato leaves. These results are of interest because TASAKI, INOUE, and MATSUOKA (10) found in connection with fertilizer experiments with satsuma oranges that the omission of phosphate brought about not only a decreased yield but also an increased acidity in the fruit. It would also be of interest to know whether an excess of phosphate would bring about increased acidity in fruit.

In table II, if we assume the non-reducing sugars as being chiefly sucrose, a relation appears to exist not only between the percentages of phosphorus in the culture solution and in the dry matter of the leaves, but also between these percentages and those of sucrose.

Analyses were made of the ash of the lemon cuttings and the data are presented in table III. The percentage of total ash in the new mature leaves was least at the 1 to 10.5 p.p.m. phosphate concentration and greatest at the lowest concentrations of phosphate (0.0125 to 0.075 p.p.m.). COLBY (2) previously found that the leaves of French prune tree cultures lacking phosphate were high in ash.

The lowest percentages of calcium and potassium in the mature new leaves were found also at the 1 to 10.5 p.p.m. phosphate concentration, while the highest percentages were found at the lower phosphate concentrations in which deficiency symptoms were evident. These results agree with those of ECKERSON (4) on tomato plants and of DAVIS (3) on fruit trees.

The total nitrogen and nitrogen (in the form of nitrate) in the dry matter of the lemon cuttings were determined by the Kjeldahl and reduction methods, respectively, and the results are shown in table IV. In the leaves and shoots the percentages of total nitrogen are

TABLE III

TOTAL ASH, CALCIUM, MAGNESIUM, SODIUM, AND POTASSIUM CONTENT OF LISBON LEMON CUTTINGS*

PO_4 IN CULTURE SOLUTION (P.P.M.)	TOTAL ASH AS PERCENTAGE OF DRY MATTER			CALCIUM AS PERCENTAGE OF DRY MATTER			MAGNESIUM AS PERCENTAGE OF DRY MATTER			SODIUM AS PERCENTAGE OF DRY MATTER			POTASSIUM AS PERCENTAGE OF DRY MATTER		
	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS
0.0125 to 0.075	13.34	6.77	8.56	2.25	1.38	1.53	0.44	0.17	0.21	0.22	0.54	0.17	4.08	2.01	1.66
0.1 to 0.5	12.14	6.51	9.12	2.33	1.33	1.36	0.41	0.10	0.23	0.21	0.48	0.19	3.27	1.88	2.16
1 to 10.5	10.34	6.05	10.52	2.06	1.21	1.28	0.37	0.12	0.30	0.14	0.19	0.22	2.72	1.65	2.64
15.75 to 31.5	10.56	7.01	8.77	2.09	1.05	0.94	0.34	0.13	0.55	0.15	0.16	0.23	2.77	1.42	2.29
105	11.07	6.32	10.74	2.11	1.13	1.13	0.36	0.14	0.51	0.22	0.26	0.70	3.05	1.78	0.78

* Cuttings grown from July 3 to December 19, 1934, in solution cultures at different phosphate levels.

greatest at the lower concentrations of phosphate, which is in agreement with the results of COLBY on French prune trees.

The percentages of nitrate nitrogen in the leaves and shoots are highest in the cuttings grown with the least phosphate. ECKERSON and others secured similar data for tomato plants. The results of these investigators and those reported in table IV show that when phosphorus is deficient the reducase activity as described by ECKERSON evidently suffers a great reduction because the absorbed nitrate

TABLE IV

TOTAL AND NITRATE NITROGEN CONTENT OF LISBON LEMON CUTTINGS*

PO_4 IN CULTURE SOLUTION (P.P.M.)	TOTAL NITROGEN AS PERCENTAGE OF DRY MATTER			NITRATE NITROGEN AS PERCENTAGE OF DRY MATTER		
	NEW LEAVES	NEW SHOOTS	NEW ROOTS	NEW LEAVES	NEW SHOOTS	NEW ROOTS
0.0125 to 0.075	3.54	1.26	2.59	2.29	0.92	2.31
0.1 to 0.5	3.62	1.12	3.11	1.65	.42	2.56
1 to 10.5	2.77	0.99	3.56	0.49	.43	2.27
15.75 to 31.5	2.60	0.98	4.01	0.36	.27	.....
105	2.75	0.32	3.10	0.44	0.53	.....

* Cuttings grown from July 3 to December 19, 1934, in solution cultures at different phosphate levels.

remains largely unchanged in the tissues. For this reason, in agreement with ECKERSON, trees grown with abundant nitrate but with deficient phosphorus are essentially nitrogen starved plants.

Summary

1. A study was made of the concentration of phosphate in the culture solution in relation to the growth and composition of Lisbon lemon cuttings. It was found essential to keep iron out of the solution during intervals in which the phosphate concentration was being maintained.

2. The cuttings were grouped into certain ranges of phosphate concentration in order to simplify the harvesting and analytical analysis.

3. Cuttings grown in solution cultures containing from 0 to 0.2 p.p.m. showed symptoms of phosphorus deficiency regardless of the frequency of change of solution. Slight symptoms of phosphate de-

ficiency occurred at 1 p.p.m. phosphate but none at 2 p.p.m. The longest roots were produced in the low range of phosphate concentration and the shortest roots in the highest range.

4. Lemon cuttings grow well in solutions containing 105 p.p.m. phosphate when such solutions are vigorously aerated. Aeration appears to be an important factor in the phosphate nutrition of citrus.

5. The percentages of phosphorus in citrus leaves in the field decrease as the maturity of the leaves increases. However, in the mature original leaves of the cuttings the percentages were increased with increasing phosphate concentrations in the culture solution.

6. The percentages of reducing sugars in the mature leaves (not the original leaves of the cuttings) are at a maximum at the phosphate concentration range of 1 to 10.5 p.p.m., while those of the nonreducing sugars increase with increasing phosphate concentration. A relation appears to exist not only between the percentages of phosphorus in the culture solution and in the dry matter of the leaves, but also between these percentages and those of sucrose.

7. Phosphate deficient lemon leaves resemble mottled leaves (which contain an excess of phosphorus) in having a greater acidity than healthy leaves.

8. The percentages of total ash, calcium, potassium, total and nitrate nitrogen in the new mature leaves were greatest at the lowest concentrations of phosphate (0.0125 to 0.075 p.p.m.). Absorbed nitrate remains largely unchanged in the tissues when phosphorus is deficient. Trees grown with abundant nitrate but with deficient phosphorus are essentially nitrogen starved plants.

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GERMINATION OF POPULUS GRANDIDENTATA AND P. TREMULOIDES, WITH PARTICULAR REFERENCE TO OXYGEN CONSUMPTION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 472

MILDRED E. FAUST

(WITH ONE FIGURE)

Introduction

Populus tremuloides Michx. and *P. grandidentata* Michx., which are known to reproduce chiefly by means of root shoots, produce seeds in great abundance in parts of the eastern and central United States. In these regions seedlings are not rare, although most of the reproduction, particularly following burns, is by means of root shoots. Where there is sufficient rainfall there are usually thousands of seedlings germinating on the old leaves or moist soil under or near the mature trees. Few if any of these ever reach more than a few inches in height.

P. tremuloides, which is one of the most widely distributed trees in the western hemisphere, as well as *P. tremula* L., a parallel species in the eastern hemisphere both as to habits and distribution, has short lived seeds which are found in abundance only in certain portions of its range. In Sweden, LAGERBERG (8) states that LOWENHJELM found difficulties in germinating the seeds since they remain viable for only three weeks. PEARSON (12), WEIGLE and FROTHINGHAM (13), and others find that seeds of *P. tremuloides* are not viable under normal conditions for more than three weeks. According to BAKER (2) and PEARSON (12), few seeds have been found in Utah. The latter has also found that in central Utah, owing to the short period of viability, it is difficult for seedlings to become established, except in locally wet places, as the surface of the soil is dry and rains are infrequent at the time of seed dispersal. This almost total absence of seedlings of *P. tremuloides* in such regions as Utah, as well as the small number which persist in other sections, raises the question as to the conditions under which germination and growth are possible.

An outstanding example which answers this question in part is

found at Strawberry Lake, Utah (altitude 7560 feet), where aspen is abundant in the adjacent hills. According to data obtained from W. P. Cottam, the reservoir was completed in 1912 and by June, 1913, 6000 acre-feet of water were impounded. In 1922 the water reached the spillway and again a year later. Since 1923 the water has never filled the lake, and today (1933) there are some 4900 acres exposed which were under water in 1923. The sagebrush which covered this shore region was killed through inundation and the barren opened to invasion. In 1928 the writer found intermingled with the dead sagebrush a great number of seedlings of *P. tremuloides*. Here the ground water of the denuded area was the important factor in establishing the aspen seedlings and supplied the deficiency ordinarily encountered in Utah where the climatic factors are the determiners.

From observation and repeated tests it is evident that seeds of both species of aspen will germinate under a varied range of environmental conditions as long as sufficient moisture is provided. Germination continues unhampered when the seeds are totally submerged in water.

Since moisture appears to be a critical factor in the establishment of these seedlings, and since the seedlings are relatively short lived, it was pertinent to study the effects upon viability of drying the seeds for varying lengths of time and thus of prolonging viability. Temperature studies on rate and percentage of germination were made on seeds of various ages and moisture contents, as well as tests of oxygen consumption at a constant temperature.

Seeds of *P. grandidentata*, which are produced in much greater abundance than those of *P. tremuloides* in the immediate vicinity of study, were used in most cases. It was found that there is practically no difference in the characteristics of the seeds of the two species. The catkin of the former is larger, being about 6 inches long and containing approximately 1000 viable seeds. It is produced a few days later than that of *P. tremuloides*, and before the leaves instead of with or after them.

The term "germination" as here used indicates a point at which the hypocotyl has grown out of the seed coat just far enough to raise one end of the seed from the substratum.

Material and methods

COLLECTING.—The seeds used for detailed studies were collected from trees growing in the crevices of the Onondaga limestone in the vicinity of Syracuse, New York. Records at Syracuse since 1922 show that anthesis for both species varies from March 19 to April 12. From 1927 to 1933 *P. tremuloides* seeds matured from May 10 to 20 and those of *P. grandidentata* from May 12 to 27.

The best time for collecting is on a clear calm day; however, seeds collected in the rain have retained 100% viability at 5° C. for at least one year. The seeds used for study were collected from catkins picked from the trees when the seeds were a light straw color. Those collected before reaching the characteristic straw color do not ripen completely and yield approximately 50% germination.

TRANSPORTATION.—The best method found for transporting seeds in the green catkins over long distances was packing them loosely in fine cheesecloth inserted in burlap bags. One lot of seeds was in transit for three days in extremely hot weather and maintained 100% viability after storage at 5° C. for two years.

DRYING.—Within an hour after collection, the catkins were spread out one or two layers thick in large shallow pans out of direct sunlight in an inside room of uniform temperature. In 1932 both an aneroid barometer and a Julien and Friez hygrothermograph were placed in this drying room. The mean atmospheric pressure for the first two weeks of drying (that is, from May 26 to June 8) was 29.92 inches. This varied from 29.60 to 30.25 inches. The mean temperature was 23.61° C. with a variation of from 20° to 26° C. The mean relative humidity of 8:00 A.M. and 12 noon was 48.50% with a range of from 27 to 66%.

The best length of duration of drying is from three to eight days. In 1930, when the largest number of seeds were collected, both species yielded 100% germination when first collected. Tests at 29° C. were made for seeds dried one, two, three, four, five, six, eight, nine, and ten days and then stored at 5° C. Germination percentages for seeds of both species are similar except that the drop in viability comes sooner in *P. tremuloides*. Those of *P. grandidentata* dried one day showed a sudden drop in germination to 47% before fifteen days and were dead in three months. Those dried both nine and ten days

were dead in thirty-six months and those dried both two and four days were dead in forty-five months. The ones dried six days showed 14% germination in forty-five months and those dried both three and five days had 44 % viable after forty-five months.

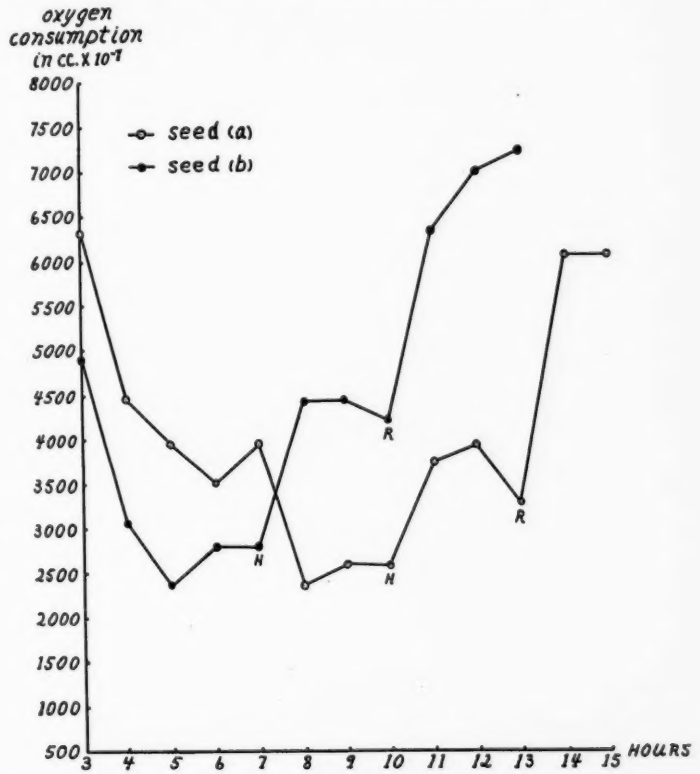

FIG. 1.—Hourly oxygen consumption of single seeds (*a*) and (*b*), *P. grandidentata* 1932, dried six days. Hypocotyl of seed (*b*) emerged two hours before that of seed (*a*), showing individual differences in germination. *H*, hypocotyl; *R*, root hairs.

CLEANING AND GRADING.—When the seeds had dried the desired length of time, the catkins were placed over a wire screen on top of an open box and were separated from the cotton by means of a heavy brush. The seeds which dropped through the screen were blown free

from chaff and graded through screens of 30 and 40 mesh to the inch. Seeds graded with the former screen were used for all the tests except as indicated later.

There is a definite parallel between the curves of germination of small and large seeds. The total percentage of germination of *P. grandidentata* 1928, which was dried four days and planted when thirteen to seventeen days old, showed an average germination of 45% for small seeds as compared with 96% for the large ones.

PLANTING.—All tests of seed germination, unless otherwise noted, were made in petri dishes containing two layers of thoroughly moistened filter paper with the excess water drained off.

The seeds will germinate whenever there is sufficient moisture. At the same temperatures the total percentages of germination are the same regardless of whether or not the seeds are totally submerged in water, or are on filter papers with varying amounts of moisture in similar or different sized dishes.

The percentage of germination was found to be comparable on filter paper (moistened with distilled water), sand of pH 7.0, and humus (in which the aspens grow) of pH 8.5. However, only 82% germinated on sand of pH 4.5 as compared with 98% on the other media.

Twenty-five to one thousand seeds were used for each germination test. In the studies of the effect of temperature upon germination fifty and one hundred seeds were used. It was found that fifty and usually twenty-five gave reliable results when the seeds were of high viability.

STORING.—The seeds were stored in three ways: (1) in open dishes in the laboratory, (2) in corked bottles in the laboratory, and (3) after 1930 in corked bottles at 5° C. in a General Electric refrigerator constant to $\pm 0.5°$ to 1° C. The seeds occupied from one-half to one-fourth the volume of the bottles, which were opened for ventilation at various intervals.

After eighteen weeks of storage both in open dishes and corked bottles in the laboratory, seeds of both species gave germination percentages of 1. However, *P. grandidentata* 1930 which had been dried both three and five days before storage in a bottle showed 1% after twenty-two weeks. The latter remained 100% viable for twelve

weeks when dried five days, for eleven weeks when dried four days, for nine weeks when dried three days, and for seven weeks when dried nine days. *P. tremuloides* 1930 dried both two and four days before storage in bottles remained 100% viable for thirteen weeks. Any seeds stored in the open retained their 100% viability for only five weeks.

After storage in bottles at 5° C., seeds dried more than two days showed the greatest viability for long periods of time. Those of *P. grandidentata* 1930 which were dried for one day only and placed in a bottle in the laboratory had 76% germination at the end of one week and 0% in two weeks, while the same lot of seeds stored at 5° C. lost viability in twelve weeks.

Until 1930 an icebox of varying temperature was used for low temperature storage. Seeds kept in it fell off more rapidly in percentage of germination than did those kept constantly at 5° C. *P. grandidentata* 1929 and 1930, germinated at 29° C. when thirty-two weeks old, showed 29% for those of 1929 and 98% for those of 1930. After one year *P. grandidentata* 1929, dried five days, gave 1% germination; while that of 1930, also dried five days, gave 91% and was 76% viable after three years.

Cold storage (about 5° C.) of fully matured and dried seed has been found to be important for extensive prolongation of vitality of short lived seeds. NAKAJIMA (**10**) in his study of *Salix* found that storage under several conditions at a low temperature prolonged vitality. In one instance he obtained 53% germination after three hundred and sixty days. JOSEPH (**6**) finds that the vitality of parsnip seeds is prolonged decidedly by air drying to a certain low moisture content and subsequent storage in an icebox with frequent ventilation.

Germination results

TEMPERATURE RELATIONS

Germination of both species is not dependent upon light, and with sufficient moisture will take place at any temperature between 0° and 35° C. The General Electric refrigerator was used for germination at temperatures of 5° C. and below and ovens constant to ±0.5° C. for temperatures of 26°, 29°, 32°, 35°, and 38° C.

Seeds dried and stored under similar conditions, even though collected from different trees and in different years, gave similar germination percentages. Seeds of *P. tremuloides* 1930 and 1932 dried three days, before storing at 5° C. for one year, showed germination of 97% at 29° C. Seeds of *P. grandidentata*, stored and germinated under similar conditions, showed 100% in 1930 and 97% in 1932.

MAXIMUM, MINIMUM, AND OPTIMUM TEMPERATURES.—No particular attempt was made to determine the maximum, minimum, and optimum temperatures for the two species. After exposure for two weeks to the highest temperature used (38° C.), no seeds germinated. When these were transferred to both room temperature and 29° C., however, a number of them were found viable.

Seeds of *P. grandidentata* 1929, dried five days before storage on ice for twenty to twenty-three weeks, gave no germination at 35° C. but gave 39% at 32° C. and 20% at 29° C. A larger number of young seeds will germinate more quickly at 35° C., but complete (100%) germination is attained sooner at 29° and 32° C. Seeds up to one year old which have been frozen for several days and thawed at 5° C., as well as those kept constantly at 5° C., germinate in five days at 5° C.

The largest percentage of germination was attained at both 29°and 32° C. However, the sturdiest appearing seedlings were those from seeds germinated at temperatures from 5° to 29° C. Seedlings of *P. grandidentata* 1929 three weeks old were twice as tall when germinated at 29° as those germinated at 35° C.

Both species of 1930 seeds which were dried for periods of two, three, four, five, and six days and studied for a period of the first five weeks after collection showed curves for the rate and percentage of germination which were practically parallel at the three temperatures, 26°, 29°, and 32° C. The rate was always slower for those germinated at 26° and the average final percentage of germination was 98 as compared with 100 for those germinated at 29° and 32° C.

INITIAL GERMINATION.—Seeds of both species collected from the same trees over a period of six years have been found to show the initial germination in five hours at 29° and five days at 5° C. Occasionally the seeds of *P. tremuloides* germinate in four hours at 29° C. It is found that when seeds are germinated at a higher temperature (29° C.) the initial time becomes greater as the seeds age. For seeds of *P. tremuloides* 1930 dried three, four, and five days and

P. grandidentata 1930 dried four, five, six, nine, and ten days, initial germination at 29° C. occurs in five hours for a period of thirty-two weeks, then it increases to seven hours at forty-nine weeks, to nine hours at one year, and from twelve to twenty-four hours at three years.

P. tremuloides seeds of the same lot as the preceding but dried two days required for initial germination only seven hours at the end of one year; while *P. grandidentata* seeds from the same lot but dried three days required seven hours at forty-nine weeks, eight hours at one year, and from twelve to eighteen hours at three years. The latter retained 100% viability for one year and dropped to 84% in three years.

Microchemistry

Microchemical tests were made on two sets of one year old seeds of *P. grandidentata* 1930 with 0% and 92% germination, on one set of *P. tremuloides* 1930 with 90%, on three week old 1931 *P. grandidentata* and *P. tremuloides* with 100%, and on *P. grandidentata* 1931, three day old seedlings.

The contents of the seeds of both species at the same age are comparable. The outer layer of the seed coat of the resting seeds is composed of cellulose and the inner of unknown (yellowish brown) material. The outer wall becomes partially broken down into sugars (mostly sucrose) and starch in the year old viable seeds and decidedly so in the dead ones. In the latter the inner wall breaks down, to a lesser extent, into sugars (some glucose) and starch.

The storage products in the two (or three) cotyledons are fats and proteins, these being in similar amounts and arrangements in the viable young and year old seeds but reduced in amount in the dead seeds.

In the seedlings the fats are scattered throughout the cotyledons but concentrated near the veins. The proteins are near the veins and at the base of the hypocotyl. Small amounts of glucose, fructose, and sucrose are scattered throughout the seedling.

The storage compounds appear to have some bearing upon germination and after-ripening. Jones (**5**) found that in the genus *Acer* the short lived seeds (*A. saccharinum*) have storage compounds composed mainly of sugars, while the comparatively long lived ones which require after-ripening (*A. saccharum*) have compounds of pro-

teins and fats. Here aspen seeds, with storage compounds of fats and proteins, are found to be short lived, at least in temperate climates. Upon submission to low temperatures, however, they become comparatively long lived.

MOISTURE CONTENT

Moisture determinations of seeds of *P. grandidentata* 1932 were made from averages of two lots of seeds of 1 gm. each which were ground fine in an agate mortar. They were then dried in a vacuum desiccator over concentrated sulphuric acid and kept in a constant temperature oven at 60° C. The moisture percentages after the seeds had been in storage twelve weeks in closed bottles at 5° C. are shown in table II.

When the seeds are heated above 60° C. there is decided discoloration, but from a few tests, sufficient only to give a suggestion, it appears that a higher temperature does not appreciably alter the percentage of moisture of those air dried between four and eight days. Although the seeds of the two species vary slightly in size, the moisture percentage is similar in both when submitted to the same drying conditions. Seeds of *P. tremuloides* 1930, which were dried at 104° C., seven months after collection showed 8.62% moisture. These had been dried for five days before storage at 5° C.

Both 1931 species were dried in a vacuum oven at 79° C. Those of *P. tremuloides*, which were dried five days before storage and the moisture determinations made after they were twenty-six days old, showed 8.83%. Those dried four days before storage and tested after nineteen days, showed 8.85% moisture. *P. grandidentata*, dried for six days before storage and tested when fifteen days old, showed 8.45% moisture. The 1931 seeds of *P. tremuloides* were 100% viable and those of *P. grandidentata* were only 50% viable.

Other writers have also found that longevity of seeds is increased by reduction in the moisture content. JOSEPH (**6**) found that for parsnip seeds, somewhere below 6.13% moisture at room temperatures appeared to be the "critical moisture content."

Oxygen consumption

A Thunberg microrespirometer, modified by FENN (1927) and later by OBRESHKOVE (OBRESHKOVE and BANTA **11**), was used for the study of the oxygen consumption of both seeds and seedlings.

The extreme sensitiveness of the apparatus is apparent by its capacity for measuring accurately and directly the oxygen consumption of ten resting seeds or a single germinating seed of *P. grandidentata*, the average weight of each being 0.0000941 gm.

Attempts were made to use KRAJNIK'S (7) microrespirometer, an apparatus first used on plants (strawberry fruits) in this country by GERHART (3). Owing to its large size, however, and the fact that it could not be entirely submerged in the water bath, it lacked the sensitivity necessary for reactions of very small objects.

The microrespirometer, which is composed of two glass bottles connected by a capillary containing a kerosene drop, was totally sub-

TABLE I

OXYGEN CONSUMPTION FOR SEEDS OF P. GRANDIDENTATA 1932

MICRORESPIROMETER TEST	OXYGEN CONSUMPTION PER SEED PER MINUTE IN CC. $\times 10^{-7}$				
	SEED 0	SEED 2	SEED 4	SEED 6	SEED 8
First hour	53.499	25.309	10.823	14.640	9.656
Second hour	52.642	19.701	14.485	14.484	14.374
Third hour	49.371	23.985	17.910	21.804	16.197
Fourth hour	48.437	25.153	20.091	20.168	19.076
Average	50.987	23.537	15.827	17.774	14.826

merged in a Freas waterbath with a thermostat control set at 25° C. and sensitive to ± 0.005° C. It was absolutely necessary to keep the temperature as nearly constant as possible. A variation of more than ± 0.01° C. caused enough fluctuation in the volume of oxygen consumed to render the results worthless. In order that the microrespirometer attain the exact temperature of the waterbath, it, with its contents of a 2% solution of NaOH and the seed or seeds, was submerged in the bath for an hour before it was closed to the air and then left another hour before readings were taken.

For resting seeds readings were taken hourly over a period of four hours (table I). The oxygen consumption is represented in cubic centimeters times 10^{-7} per seed per minute. Seed 0 is the lot of seeds not dried at all, seed 2, that dried two days, etc. The recorded tests are from those lots of seeds which showed 100% germination after removal from the microrespirometer. Care was taken at all times to

handle the seeds as little as possible when removing from storage and placing in the microrespirometer.

The seeds used were those of *P. grandidentata* 1932 which had been dried zero, two, four, six, and eight days. Ten seeds were used and an average of five tests is recorded for each hour. Repeated tests at the immediate close of the drying period and also within two months after they were stored showed comparable amounts of oxygen consumption.

As the moisture content of the seeds decreases the oxygen consumption decreases (tables I and II). Such a correlation has been found for certain other seeds by a number of workers, notably BAILEY (1), JAQUOT and MAYER (4), and MAIGE and NICOLAS (9).

TABLE II

OXYGEN CONSUMPTION AND MOISTURE ABSORPTION FOR SEEDS OF P. GRANDIDENTATA 1932 DRIED FOR DIFFERENT LENGTHS OF TIME

No. of days dried	Average 4 hours oxygen consumption	Percentage moisture absorbed	Moisture content	Percentage germination at tests	Percentage germination at end of one year
0	50.987	.....	49.22	100	0
2	23.537	.....	30.52	100	0
4	15.827	11.30	9.18	100	67 (poor)
6	17.774	12.07	6.65	100	95 (healthy)
8	14.826	10.02	7.52	100	95 (poor)
73	.....	.....	6.97	100	.....

As noted in table I, there is a gradual increase in the amount of oxygen consumed during the first four hours of the tests of seeds 4, 6, and 8. It was found that these absorbed an appreciable amount of water from the NaOH solution (table II).

The comparatively large amount of oxygen consumed in freshly harvested seeds drops to approximately half after one day of drying. After the third day of drying there is no considerable difference in the amount consumed. Of the 1932 seeds those dried six days are the best from the standpoint of longevity, for after thirty-two months 85% of them were viable as compared with 65% of seed 8 and 17% of seed 4.

The most striking results were obtained when the oxygen consumption of a single seed placed on moist filter paper in the micro-

respirometer was checked at five-minute intervals. The seedlings were all taken from seed 6 which had been in storage at 5° C. for two months.

In order to obtain a relative idea of the oxygen consumed during the first two hours, a seed (*c*) was placed in the microrespirometer for only fifteen minutes before readings were taken. Using round numbers for cubic centimeters of oxygen times 10^{-7}, during the second fifteen minutes it absorbed 13,300; during the third fifteen minutes, 4200; and the fourth fifteen minutes, 1700. Two other seeds (*a*) and (*b*) were left in the microrespirometer for one hour before it was closed and the readings taken for the second hour of germination. Seed (*a*) consumed approximately 47,200 and (*b*) 41,100 cc. times 10^{-7}. Figure 1 illustrates the results of the latter two seeds from the third to the fifteenth hours of germination and the third to the thirteenth hours respectively. Here the results are noted after the seeds and apparatus had been in the bath for two hours, as was done with the resting seeds. These two seeds were chosen because they illustrate individual differences in initial time of germination. The hypocotyl of seed (*b*) was 1 mm. long and the crown of root hairs 0.5 mm. long (one-half final length) at the end of thirteen hours, while it required fifteen hours for those of seed (*a*) to attain the same lengths.

Summary

1. *Populus tremuloides* and *P. grandidentata* produce seeds in great abundance in central and eastern United States. The former seeds mature approximately three days earlier than the latter. From six to ten weeks elapse between anthesis and maturation of the seeds.

2. Seeds collected when a light straw color and dried at once retain their viability the longest.

3. The best length of time for drying is from three to eight days in a room of uniform temperature of about 24° to 25°C. Seeds dried only one day lose viability in thirteen weeks, even when placed at 5° C.

4. Small seeds do not germinate so readily nor retain their viability so long as large ones. This applies to individuals within the species.

5. Seeds showed the highest percentage of germination in media of pH 7.0 to 8.5.

6. Except when the viability is low, fifty seeds are sufficient for showing accurate percentages of germination.

7. A constant low temperature (approximately 5° C.) was found best for prolonging viability.

8. Seeds have been found to retain 100% viability for as long as thirteen weeks when placed in corked bottles in the laboratory and for five weeks in open dishes. Most of these lose their viability after eighteen weeks but occasionally show 1% at twenty-two weeks.

9. Seeds germinate whenever there is sufficient moisture (even when submerged) between temperatures of 0° and 35° C. Freezing for several weeks does not appear to injure the seeds when they are thawed at 5° C.

10. The largest percentage of germination is obtained at 29° and 32° C.; 35° to 38° C. appears to be too high but may only retard germination. The sturdiest seedlings are from seeds germinated at temperatures from 5° to 29° C.

11. The time for initial germination is usually five hours for seeds germinated at 29° and 32° C. This increases as the seeds age. Short initial germination time is correlated with high viability.

12. The seeds of both species are fatty and contain some proteins.

13. Seeds lose moisture rapidly during the first two days of drying. After three days there is little loss.

14. Oxygen consumption was studied by means of a microrespirometer of the closed system so sensitive that a temperature constant to ± 0.005° C. was necessary for accurate results.

15. The extreme sensitiveness of the apparatus makes it possible not only to measure the oxygen consumption of ten resting seeds at half-hour intervals, but also that of a single germinating seed at five-minute intervals.

16. Oxygen consumption is high when the seeds are viable and contain much water. This decreases with the decrease in water content of the seeds.

17. The very great amount of oxygen consumed when the germinating seed first absorbs water drops to a minimum two hours before the hypocotyl emerges from the seed coat. It then rises rather abruptly for an hour, drops slightly in the next two hours, after which the crown of root hairs emerges. There is again a rather abrupt rise for an hour and then it becomes comparatively uniform.

The writer wishes to acknowledge the aid of Professors HENRY C. COWLES and CHARLES A. SHULL throughout the progress of this work. Acknowledgment is also due to Professors GEORGE D. FULLER and SCOTT V. EATON for their helpful suggestions and interest and to Professor VASIL OBRESHKOVE for his kindness in lending the microrespirometer and for helpful suggestions in the determination of oxygen consumption.

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CHROMOSOME BEHAVIOR IN AGROSTIS NEBULOSA

FRED W. TINNEY

(WITH TWENTY-FOUR FIGURES)

Introduction

One of the most important regularities of chromosome behavior is the side-by-side alignment of their homologous parts during the heterotypic prophases. Thus in a typical diploid, individual meiotic chromosomes associate in pairs; in a polyploid or polysomic, the existence of more than two homologous chromosomes commonly leads to multivalent associations. Extensive cytological and genetical studies of structural changes in chromosomes, particularly of reciprocal translocations of parts of non-homologous chromosomes in several genera, confirm the general rule that homologous parts tend to pair in meiosis.

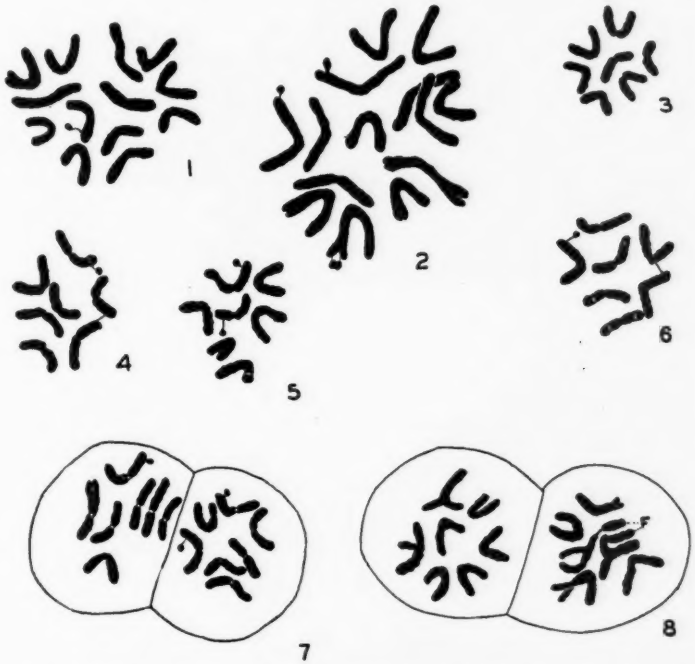
The behavior of chromosomes having reciprocal translocated segments, as shown by cytological observation, is in general the formation in late diakinesis of an open ring or chain of four or more chromosomes joined by terminalized chiasmata. The random distribution of the catenated chromosomes in metaphase I results in a chromosomal deficiency in a certain proportion of the spores, and in the great majority of reported cases such a deficiency is lethal to the gametophyte.

Little is known from previous accounts of the cytology of *Agrostis*. In 1928 AVDULOW (see **12**) found forty-two chromosomes in root tips in *A. alba* and twenty-eight chromosomes in those of *A. vulgaris*.

Investigation

For this study, seed of *Agrostis nebulosa* Boiss. & Reut. was obtained from Vaughan's Seed Company, Chicago, Illinois, and grown in the botanical greenhouses at the University of Wisconsin. Sax's modification of Navashin's fluid gave the best fixation for cytological observation. Sections of flowers and root tips were cut 10 μ in thickness and stained with Heidenhain's iron-alum haematoxylin.

The fourteen somatic chromosomes are shown in figures 1 and 2. Differences in length and form are not as yet sufficiently determined



FIGS. 1–8.*—Figs. 1, 2, equatorial plates of root tips; figs. 3–8, same of homoeotypic divisions: Fig. 1, thirteen chromosomes; one chromosome with a subterminally attached fragment (one chromosome not shown). Fig. 2, fourteen chromosomes; three chromosomes each with an attached fragment. Fig. 3, seven chromosomes; no fragments. Figs. 4–6, seven chromosomes; two chromosomes each with an attached fragment (one fragment proper is hidden in figs. 4, 6). Fig. 7, sister cells; one cell has one, the other two chromosomes each with attached fragment. Fig. 8, sister cells; seven chromosomes in one cell, in the other six and two fragments (*f*), one being attached to end of another chromosome. ×3662.

* All figures drawn with the aid of an Abbé camera lucida at table level. A Zeiss 2 mm. apochromatic objective, N.A. 1.3, and a Spencer 30× compensation ocular were used.

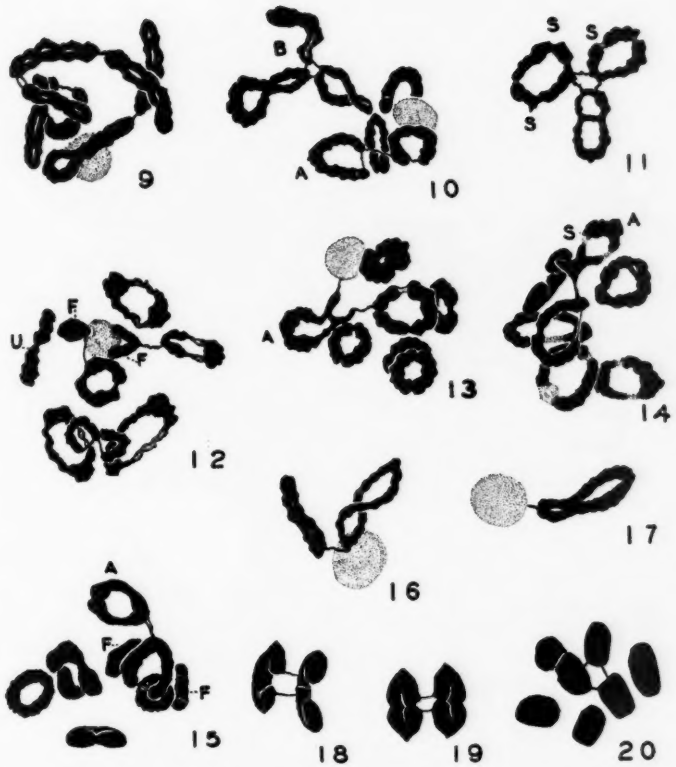
to identify each chromosome pair. However, small differences in length and in the position of spindle fiber attachments may, after detailed study of many figures, make such identification possible.

The morphology of the chromosomes is most easily observed in the homoeotypic metaphases (figs. 3–7), although in these figures the chromosomes are smaller than in mitoses in root tips. In general, three chromosomes of the haploid complement are slightly shorter than the other four; of the three shortest chromosomes two have arms of slightly unequal length. The other five (one short, four long) apparently have arms of equal length. No satellites of the ordinary type have been observed. The laterally attached structures, of which there are three in the root tips of some plants (figs. 1–2), and in homoeotypic divisions (figs. 4–7), are probably derived from chromosome fragments. They show no relationship to the nucleolus.

In this paper only the chromosome behavior from diakinesis onward will be considered, except as certain evidence presented by diplotene stages may aid in interpreting configurations in diakinesis.

Most microspore mother nuclei in late diakinesis present a typical condition of seven bivalents, each composed of two chromosomes associated by terminal chiasmata. In relatively few nuclei, although in a sufficient number to remove any doubt of the validity of the observation, two or three bivalents, usually typical in appearance, are associated together at their ends. Two types of such associations are observed—either a chain of two or three bivalents, or an association of three bivalents joined at a common point. Both types may occur in the same nucleus.

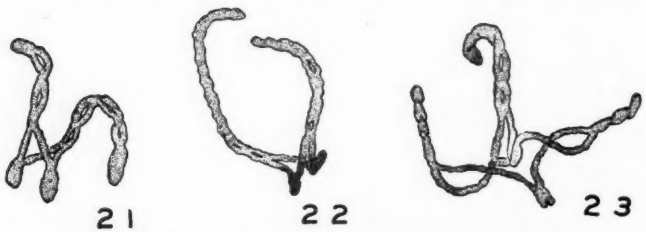
In early diakinesis the two components of each of the seven pairs are associated regularly by interstitial chiasmata (assuming that nodal points in at least some cases represent chiasmata). In some instances a variable number of the regularly formed bivalents are joined end to end in one or two chains. In the nucleus shown in figure 9 there are two chains, one consisting of three and the other of two bivalents. Each of the nuclei shown in figures 13 to 15 (*a*) contains one chain of two bivalents. Occasionally (fig. 13, *a*) one free arm of one pair is attached to the nucleolus, the corresponding free arm being joined to the end of one chromosome in another chromosome pair. The relation of the attached chromosomes to the nucleolus is not clear. In some nuclei one end of a chromosome pair is either connected by a fiber with the nucleolus (fig. 17) or is lying over the latter. In one nucleus, of which two chromosome pairs are shown in



FIGS. 9–20.—Figs. 9–17, diakinesis; figs. 18–20, equatorial plates of heterotypic divisions: Fig. 9, early diakinesis; two chains of bivalents, one consisting of three, the other of two bivalents; two bivalents are separate. Fig. 10, one tri-association (*b*) and one chain of two bivalents (*a*); two bivalents are separate. Fig. 11, three bivalents in tri-association; interstitial chiasma in one pair; spindle fiber attachment (*s*). Fig. 12, six bivalents, one univalent (*u*), two fragments (*f*). Figs. 13, 14, one chain of two bivalents (*a*), five separate bivalents; interstitial chiasma in fig. 13 (*a*). Fig. 15, one chain of two bivalents (*a*); two unattached fragments (*f*). Fig. 16, one chain of two bivalents; one pair associated with nucleolus. Fig. 17, pair associated with nucleolus. Figs. 18, 19, side view of two pairs showing respective ends connected. Fig. 20, polar view of tri-association. ×4500.

figure 16, the ends of one pair are lying over the nucleolus and also are connected to the end of a chromosome of the other pair. It is possible that the chromosome pairs here concerned are the same as those shown somewhat similarly arranged in figure 13 (*a*).

The second type of configuration (figs. 10, *b*, 11) consists of three bivalents so arranged that the corresponding ends of both chromosomes of one pair are associated respectively with the ends of chromosomes of two other pairs. For convenience this type of configuration will be referred to as a tri-association. A tri-association (*b*) and



FIGS. 21–23.—Diplotene strands which may lead to bivalent association shown in diakinesis: Figs. 21, 22, two bivalent strands; free ends of one conjugated with free ends of other. Fig. 23, three bivalent strands; free end of one chromosome of pair conjugated with one free end of one chromosome of second pair, the free end of the other chromosome conjugated with free end of one chromosome of third pair. ×4500.

a chain of two bivalents (*a*) may occur in the same nucleus (fig. 10). It is possible that in some nuclei two of these pairs (instead of three) are united, thus forming a chain of two rather than a tri-association.

The study of diplotene figures reveals a condition of pairing which by subsequent terminalization of chiasmata could result in the diakinetic conditions just described. Each thread is clearly double, the pairing in most cases being complete along the entire length. In a few nuclei observed in diplotene, two bivalent threads were regularly paired on both sides of the median spindle fiber attachment region except for the extreme portion at one end. The "free" ends of one diplotene thread were conjugated with the "free" ends of the other (figs. 21, 22). Such a condition might be expected to lead to a chain of two bivalents in diakinesis.

In one nucleus (fig. 23) three bivalent strands with free ends were seen so arranged as to produce in diakinesis a tri-association. Of the

two free ends of a given pair, one was conjugated with a free end of the second pair, the other with a free end of the third pair.

It is evident that the interstitial chiasmata terminalize on both sides of the attachment region in each pair. In some instances, however (figs. 11, 13, *a*), one chiasma in one of the catenated bivalents had apparently failed to terminalize, whereas in the other bivalent or bivalents terminalization was complete. In many bivalents, both in catenated and in separate pairs, a completely terminalized chiasma is pulled out into a chromatic thread. Since there are interstitial chiasmata regularly on both sides of the fiber attachment region, terminalization could not result in an open ring of four chromosomes without the rupture of a chromatid, which our present knowledge of chromatid behavior renders improbable. The conjugation of the free ends of the two or three bivalent threads probably does not result in a chiasma; it persists, however, in consequence of the formation of a common matrix about the conjugated ends. The force leading to terminalization within each pair pulls the free ends apart; the resultant connection is a fiber having its origin from the matrix.

BEADLE (1) has shown that translocated portions of homologous parts of *Euchlaena* and *Zea* chromosomes conjugate in zygotene and are associated after diplotene by no apparent chiasmata. He suggests that post-diplotene association may be due to the common matrix of the two chromosomes, and that, following repulsion, the daughter chromosomes are held together at their ends by a fiber originating from the matrix.

Another peculiarity of chromosome behavior in *Agrostis nebulosa* is the occasional occurrence of univalents and fragments. In one nucleus (fig. 12) there were six typical bivalents, one univalent (*u*), and two fragments (*f*). Each fragment was joined to the end of a chromosome of a different pair. The two fragments taken together probably represent the homologue of the univalent. Another nucleus (fig. 15) contains six pairs and two unattached univalents (*f*). Occasionally in metaphases of homoeotypic divisions fragments of a similar nature occur. In the sister cells (fig. 8) one cell has seven chromosomes; the other six and two fragments (*f*), one of which is attached to the end of another chromosome.

In equatorial plates and in metaphases, the chromosomes lie too

closely together for multivalent associations to be readily observable; in many instances too the connections are probably broken by this time. However, figures 18 and 19 show two instances in each of which two neighboring pairs are attached by fibers terminal to their individual chromosomes. A polar view of a tri-association in the equatorial plate stage is shown in figure 20.

The seven pairs of chromosomes are regularly arranged at the equator of the spindle. The connections between the pairs observed in diakinesis, if they now exist, do not affect chromosome disjunction.

In the metaphases of homoeotypic divisions and in the mitoses in root tips an occasional chromosome bears a subterminally attached fragment. In one plant studied in detail three such chromosomes were the largest number observed in any root tip nucleus; it is possible, however, that fragments borne on other chromosomes were hidden. In the same plant, homoeotypic metaphases occasionally show a total of three chromosomes, each with an attached fragment, on two sister spindles. In some sister cells (figs. 4–6) there are two "fragment" chromosomes (one fragment proper is probably hidden in cells shown in figures 4 and 6); in others there is one; and in others, none (fig. 3). In the two sister cells shown in figure 7, one cell has one fragment chromosome, the other has two. If three such chromosomes are present in the spore mother cell, a 0–3 or 1–2 distribution would be expected to occur in the heterotypic division. In some plants no fragments have been observed; and in one plant only one chromosome could be found with an attached fragment.

Laterally attached fragments have been reported previously in *Tradescantia* (**5**), *Allium* (**9**), and *Festuca elatior pratensis* (**10**).

Several hundred microspores and pollen grains in different stages of development were studied and in no case was an irregular nuclear or cell division observed. A count of the mature pollen grains showed about 3 per cent abortion.

Discussion

Association in meiosis of four or more "non-homologous" chromosomes has been described in a number of genera, including several forms of the Gramineae (*Zea mays*, **2**, *Briza media* and *Anthoxanthum*

odoratum, **7**, **8**, *Festuca pratensis*, **10**). In nearly every case thus far reported the chromosomes are joined terminally into a ring or chain. In those cases of this nature which have been studied sufficiently to present cogent evidence on the basis of the hypothesis of segmental interchange, at least half of the chromosomes in the ring (one of each pair) have each a relatively large interchanged segment. Alternate disjunction of such catenated chromosomes results in the production of complete chromosome complements, and hence in the viability of the spores containing them; adjacent disjunction produces some chromosome combinations that are non-viable because of a deficiency of a relatively large part of a chromosome. It has been shown in *Zea mays*, however, that a small deficiency may be non-lethal or only partially lethal to the gametophyte (**3**, **4**, **11**).

HÅKANSSON (**6**) found in *Pisum* associations of two bivalents in which the chromosomes of one pair were connected by two terminal chiasmata, those of the other pair by one terminal chiasma. He suggests that in such cases small terminal portions of certain chromosomes had been translocated.

As has been seen, conditions of chromosome pairing appear at diplotene in *Agrostis nebulosa* from which both types of bivalent association herein described might be derived. In reference to the chain configurations, the diplotene strands in question are composed each of two chromosomes paired for the greater part of their length in consequence of interstitial chiasmata. The free end of each chromosome is paired with a free end of a chromosome of another bivalent strand (figs. 21, 22). Assuming that only homologous parts of chromosomes pair, and that as in *Zea* the homologous parts conjugate along their entire length, the behavior of these strands leads to the assumption that the terminal portions of two chiefly non-homologous chromosomes had been involved in a reciprocal translocation. There was no evidence of chiasmata in the free arms; apparently they were associated in consequence of their homology until a common matrix formed about them; or if there was a chiasma it had slipped off the end, pulling out the matrix into a fiber. A possible interpretation of this nature based on segmental interchange is shown in figure 24, *A*.

In other diplotene figures three bivalent strands are associated at a common point (tri-association, fig. 23). The end of one chromosome of a pair conjugates with the end of one chromosome of a second pair; the corresponding end of the latter conjugates with the end of

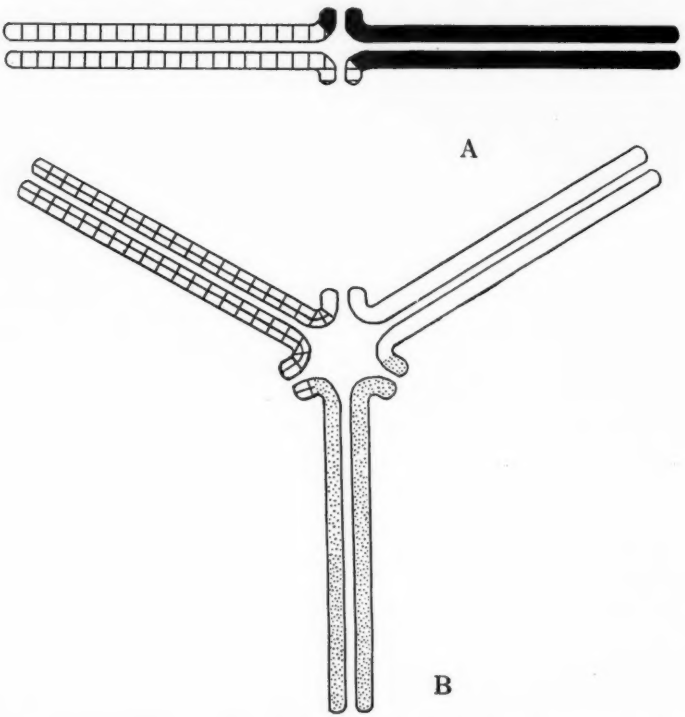


FIG. 24.—*A*, suggested constitution of the two chromosome pairs found in chain at diakinesis; *B*, suggested constitution of the three chromosome pairs found in tri-association at diakinesis.

one chromosome of a third pair. No chiasmata were probably formed in the free ends which apparently remain held together, as in the previous case, by a common matrix. The presumably homologous parts of the chromosomes and their translocations are shown in figure 24, *B*. It is evident that, if the interpretation here indicated

is correct, this is a three-way translocation (tri-translocation). To account for such translocation it is conceivable that the ends of the three non-homologous chromosomes were at some time accidentally in contact, and that in consequence of a twisting torsion the end of each was broken and became attached to one of the other two non-homologous chromosomes.

The subterminally attached fragments observed in the equatorial plates of the homoeotypic and of root tip divisions may in some way be related to the translocation here suggested. The fact that three was the greatest number of fragment chromosomes found suggests the possibility, for which there is no available proof, that these three chromosomes and their respective homologues are the ones found associated in the tri-association and that the fragments are the translocated chromosome parts. It remains possible, however, that the fragments are related to the reciprocal translocation which involved at least two other chromosomes, or that the fragments resulted from an irregular separation of daughter halves of chromosomes and bear no relationship to either of the multivalent associations.

If we assume that reciprocal translocation and tri-translocation have taken place, it is evident that the deficiencies resulting in some microspores are not lethal to the gametophytes. The small amount of aborted pollen observed might well be found in a typical individual.

Summary

1. The haploid chromosome number in *Agrostis nebulosa* is seven.

2. In most instances seven typical bivalents appear in diakinesis, the chromosomes of each pair being connected by two terminalized chiasmata. In a few instances two or three bivalents are joined end to end in a chain; or three bivalents are joined at their ends at a common point (tri-association); both types may occur in the same nucleus. Occasionally univalents and fragments are found.

3. Each diplotene strand is clearly double, the pairing in most cases being complete along the entire length. In a few nuclei observed in diplotene, two bivalent threads were regularly paired on both sides of the median spindle fiber attachment except for a very short portion at one end. The free ends of one diplotene thread were

conjugated with the free ends of the other. Such a condition might be expected to lead to a chain of two bivalents in diakinesis. In one nucleus three bivalent strands with free ends were seen so arranged as to produce in diakinesis a tri-association.

4. Interstitial chiasmata usually terminalize in typical and catenated bivalents on both sides of the spindle fiber attachment. In a few catenated bivalents complete terminalization was not attained. In no case observed was there an open ring of four or more chromosomes in diakinesis in consequence of terminalized chiasmata.

5. It is suggested that a small terminal portion of one chromosome in each of two or three pairs found in a chain has undergone reciprocal translocation, and the extreme end of one chromosome in each of three other bivalents found in a tri-association has undergone a three-way translocation.

6. Laterally attached fragments seen in somatic and homoeotypic divisions are probably constant features of these chromosomes, and may bear some relationship to the translocations here suggested.

7. No appreciable abortion of pollen grains was found.

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REGENERATION OF PODOSTEMON CERATOPHYLLUM[1]

BAYARD L. HAMMOND

(WITH NINETEEN FIGURES)

Introduction

Probably no family of the angiosperms exhibits greater capacity for regeneration than do the Podostemonaceae. This capacity has become evident during a study undertaken to ascertain to what extent regeneration occurs in each of the vegetative organs of *Podostemon ceratophyllum* Michx. WARMING (2) figured several roots which had evidently regenerated their tips, and also a hapteron (organ of attachment) which had regenerated its tip; but since he used preserved material, he was apparently unable to trace the exact origin of these regenerated structures. The present study has shown that this process may occur not only in the root but also in the stem and leaf.

Podostemon ceratophyllum (figs. 2, 4) is an alga-like, aquatic dicotyledon found on rocky or gravelly bottoms of rapids in streams of North America east of the Mississippi River. Its roots are plagiotropic, dorsiventral in structure, and chlorophyll-bearing. The shoots are also dorsiventral in structure and they arise acropetally from the root, usually in pairs a short distance back of the root growing point. The roots attach themselves to the substratum by root hairs and by fleshy disk-like or finger-like haptera. The latter arise exogenously from the ventral side of the root, each under the base of a shoot (fig. 2).

The anatomy of the plant differs in several respects from that ordinarily exhibited by dicotyledonous plants. In the root the central cylinder lies nearer the ventral side, and is composed of two xylem strands, lying side by side, with poorly developed phloem above each. There is no differentiated endodermis, and the primary meristem layers are therefore not clearly defined. The same is also true of

[1] Botanical contribution from the Johns Hopkins University, no. 132.

the stem, in which its two or three bundles are not arranged in a cylinder but in a crescent-shaped row. Each leaf possesses in its petiole a single bundle of elongated cells. For further information, see HAMMOND (1).

Investigation

CULTURE METHODS

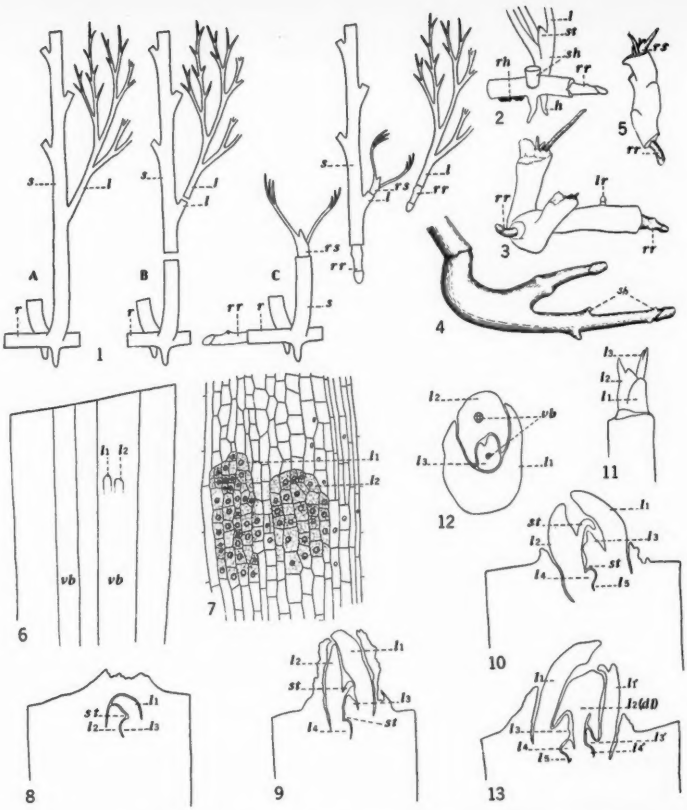
The plants seem to require constantly and rapidly moving, well aerated water, and to the lack of this may largely be attributed their failure to thrive in laboratory culture. WENT (3) reports complete inability to grow any species artificially. As it was found desirable to grow parts of the plant in the laboratory, the following four methods were tried, with the problem of aeration kept in mind.

1. Stones on which the plants were growing were placed in a jar of water through which a stream of air bubbles was supplied from an aerating system. This system is one often employed in work of this type, using the discharge from an aspirator to force air into a jar of water. Although the plants showed signs of growth, various algae soon collected in the culture and this method was consequently discontinued.

2. Pieces of stem and root were placed on wet filter paper in petri dishes, where they were kept moist and had free access to the gases of the air. Although many of the fragments regenerated and continued to grow for more than a month, the regenerated parts were weakly developed and this method was not very satisfactory, although it is convenient for studying early stages.

3. Plants grown in the greenhouse were placed in wire-screen trays under a fine spray of tap water. Here even pieces of the leaves regenerated. The regenerated parts began to appear in about four days on all organs and became about as robust as they do in their natural environment. New root growing points continued to develop on the regenerated roots indefinitely, but the leaves of the shoots from such roots always died back in a few weeks.

4. The most effective method was that of placing the plants, in wire-screen trays provided with covers, back in the stream by the undisturbed plants, when they grew just as did the latter. Because of the inconvenient distance of the stream from the laboratory, the third method was most often employed.



FIGS. 1–13.*—Fig. 1, diagram of portion of plant showing relation of regenerated parts: *A*, before portion is cut; *B*, after cutting; *C*, after regeneration of organs has taken place. Fig. 2, sketch of portion of plant showing regenerated root. ×2½. Fig. 3, showing roots which have regenerated at both ends of original root fragment. ×3½. Fig. 4, portion of root which has regenerated two laterally concrescent roots; swellings represent paired shoots which have not yet emerged. ×3½. Fig. 5, stem fragment which has regenerated a shoot at its distal end and a root at its proximal end. ×3½. Fig. 6, camera lucida sketch of median longitudinal section of distal end of stem fragment showing location of meristem from which first two leaves have been initiated. ×22. Fig. 7, detailed drawing of meristem shown in fig. 6. ×150. Fig. 8, camera lucida sketch of longitudinal section of stem showing origin of third leaf. ×22. Fig. 9, same of longitudinal section of stem fragment showing origin of fourth leaf. ×22. Fig. 10, same of longitudinal section of stem fragment showing origin of fifth leaf. ×22. Fig. 11, sketch of young regenerated shoot showing form and position of first three leaves. ×5. Fig. 12, camera lucida sketch of transverse section of young regenerated shoot showing position of first three leaves (no vascular tissue in first leaf). ×33. Fig. 13, same of longitudinal section of stem fragment showing dichotomous branching of regenerated shoot. ×22.

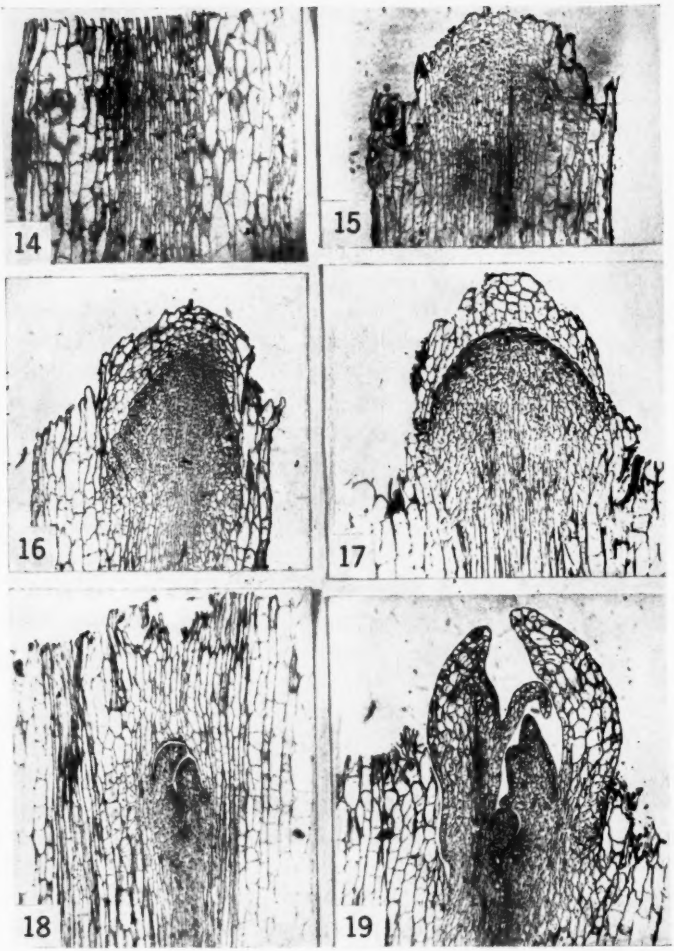
* Abbreviations: *dl*, dithecous leaf; *h*, hapteron; *l*, leaf; *r*, root; *rc*, root cap; *rh*, root hairs; *rm*, root meristem; *rr*, regenerated root; *rs*, regenerated shoot; *s*, stem; *sh*, shoot; *st*, intrapetiolar stipule; *vb*, vascular bundle.

It was found that root fragments produced roots only, while stem or leaf fragments produced both roots and shoots (fig. 1).

Regeneration from root

Pieces of root of various lengths were cut off and cultured. Samples for fixation and detailed examination were taken each day for ten or twelve days. Within four or five days a bulge appeared at the distal cut surface of each piece. This bulge was the beginning of the regenerated root which would have continued the axis of the original piece. Although a new root was always formed at the distal cut end (fig. 2), several cases of heteromorphism have been observed in which regeneration had also occurred at the proximal cut end (fig. 3), thus reversing the original polarity at this end of the piece.

The new root growing point is initiated just below the cut surface of the original root fragment. This is true of a fragment from any part of the parent root. The tissue of the central cylinder and the inner cells of the cortex about the central cylinder give rise to a meristem, which in turn gives rise to the growing point and root cap of the regenerated root. A longitudinal section of this region at such a stage shows all the cells of these tissues, except the tracheids, dividing by walls transverse to the root from the cut surface to a depth of about 0.5 mm. The earliest stages, however, show only scattered divisions (fig. 14). The cells of the new meristem continue to divide by transverse walls, and, by their subsequent growth and divisions, the meristematic mass begins to push out at the cut surface (fig. 15). In the meantime the apical growing region of the root is differentiated. This is formed at a distance of several cells from the cut surface (fig. 16). This region differs from that anterior to it by its smaller and more actively dividing cells. The cells of this anterior region are larger and less active than those beneath, and often include disintegrating tracheids of the original root. These anterior cells form the root cap (fig. 16). As growth of the regenerated root continues, the cells formed posterior to the growing point begin to elongate and soon the tissue layers of the body of the new root become differentiated. Its dorsal and ventral sides always correspond with those of the root which gives rise to it. The diameter of the root after it has emerged soon equals that of the original root, principally by the en-



FIGS. 14–19.—Fig. 14, longitudinal section through distal end of root fragment showing origin of meristem, which would have given rise to a new root growing point and root cap. ×60. Fig. 15, longitudinal section through distal end of root fragment showing origin of new root growing point and root cap. ×60. Fig. 16, sagittal section through distal end of root fragment; new root growing point and root cap clearly differentiated. ×60. Fig. 17, longitudinal section of proximal end of stem fragment showing origin of regenerated root. ×60. Fig. 18, longitudinal section of distal end of stem fragment showing origin of regenerated shoot. (This is a photograph of a similar section of same stage shown in fig. 8, except that the intrapetiolar stipule of the second leaf had not yet developed.) ×60. Fig. 19, photograph of section as shown in fig. 10. ×60.

largement of its cortical cells, thus leaving only a more or less inconspicuous collar of withering outer cells at the cut end of the old root.

Bifurcated roots are rather common in the field and often appeared in laboratory cultures (fig. 4). Although the exact mode of origin of such bifurcations was not ascertained, it is probably due to two root growing points instead of one originating at the broken end of a root; in fact two young regenerated roots are often found at the end of a broken root. It is then possible in cases of bifurcation that the tissue between two such growing points follows the growth of the latter, thus forming two roots which are laterally concrescent for a few millimeters or for a decimeter or more before the growing points become distinct (fig. 4). A transverse section of such a structure shows two cylinders. Four roots have occasionally been observed to diverge from an unusually broad root. Indications are that they are derived from two broken off, laterally concrescent roots from four fused growing points that arose at the broken end. In all observed cases of concrescence a collar is present basal to this, showing that regeneration of a broken root has taken place.

No shoots have been observed to arise at the cut or broken end of a root.

Regeneration of root from stem

The material found most satisfactory in this study was that of a large form growing at Difficult Run. The stems of this form are thicker (about 2 mm. in diameter) and the stem internodes are much longer than those of the ordinary forms, often attaining 15 mm. or more in length. Pieces of stems, 2–20 mm. or more long, were treated like the root pieces. Within four or five days a bulge appeared near the center of the proximal cut end, and a root soon appeared, always with its axis continuing that of the stem. Although out of its normal position with relation to the stem, the dorsiventral plane of the regenerated root corresponds to that of the stem. Before imbedding in paraffin, the basal end of each piece (about 5 mm. long) was cut off obliquely in such a manner that it could be properly oriented with respect to the plane of the bundles.

Within a day or two after the pieces were put in culture, the cells near the cut surface began to divide, usually by walls transverse to the axis of the stem. At first these divisions are scattered throughout

this part of the stem tissue, especially in and about the bundles. Then follows a period in which divisions occur in all planes, accompanied by a bulging at the cut surface, owing to the growth of the meristematic region beneath. This meristematic region extends from the cut surface to about 0.5 mm. or more below. Shortly after it begins to bulge, it becomes differentiated into two distinct regions, the outer one differing from the inner in that its cells are larger and the cytoplasm is less dense with smaller nuclei (fig. 17). This outer region, several cells in depth, develops to form a dorsiventral root cap. In the tissue of the meristematic region underlying this, the growing point of the root proper is initiated. Further growth of this root regenerated from a stem corresponds to that of one regenerated from a root.

Regeneration of shoot from stem

The stem fragments used were the same as those employed in the study of regeneration of a root from a stem, except that in this case the distal ends were studied. Soon after the cut surface of the distal end begins to bulge, the young leaves make their appearance (fig. 5). This is accompanied by the rupture of the tissue at the surface. Before imbedding in paraffin, just as in the case of the basal ends, these distal ends were cut diagonally for proper orientation, as the dorsiventrality of the young regenerated shoot also corresponds to that of the parent shoot.

The meristematic region which gives rise to the regenerated shoot is formed within one of the bundles at a distance of about 0.5–0.75 mm. from the distal cut surface. In this region, as may be seen in a longitudinal section, the elongated cells of the bundle begin, within a day or two after the cut is made, to divide by transverse walls. They continue to divide in the same plane until definite longitudinal rows of small isodiametric cells are formed. At first the small cells of these longitudinal rows have approximately the same transverse diameters as those of the original rows. Soon cells near the center of this meristematic region begin to enlarge and become distinguished from the others by their larger nuclei and denser cytoplasm. These cells, still arranged in longitudinal rows, form two groups, each having the general shape of a young leaf, and together they suggest the pair of cotyledons of a dicotyledonous seedling (figs. 6, 7). The shape

of each of the first two leaves of the regenerated shoot soon becomes distinct; that is, each becomes sharply delimited, except at its base, from the surrounding stem tissue. The two are usually unequal in size, and often one arises before the other. The plane joining these two leaves always corresponds to that passing through the two ranks of leaves of the original shoot. In the meantime the elongated cells of the remainder of the bundle, above the primary leaves, divide by transverse walls (fig. 7). These divisions occur progressively from the young meristematic region toward the distal cut surface of the stem.

From these first two leaves the regenerated shoot arises. There is no stem growing point. Once the leaves are fully initiated within the meristematic region, they begin to enlarge by the growth and the division of their cells in all planes. Each leaf becomes surrounded by a distinct layer of cells, the epidermis, and each pushes upward and in so doing ruptures the tissue surrounding it except at its base. The first leaf is easily distinguished by its larger size (fig. 7) and by the character of its subsequent development. On the inner surface near its base a protuberance nearly half the length of the leaf itself is then initiated by the growth and divisions of the cells beneath the epidermis, accompanied by growth and anticlinal divisions of the cells of the latter. This protuberance is the rudiment of the third leaf (figs. 8, 18). On the inner surface of the second leaf, just above the third leaf rudiment, there is formed the rudiment of the intrapetiolar stipule of the second leaf (fig. 8). The portion of this leaf above the rudiment of the intrapetiolar stipule develops to form its blade. By further growth the intrapetiolar stipule of this second leaf comes to inclose the rudiment of the third leaf. As growth proceeds, the rudiment of the fourth leaf is initiated at the base of the second leaf (fig. 9). At this time the rudiment of the third leaf begins to differentiate into a blade and intrapetiolar stipule. This stipule incloses the rudiment of the fourth leaf just as that of the second leaf incloses the rudiment of the third leaf. The fifth leaf (figs. 10, 19) and all subsequent leaves arise in the same manner as the third and fourth leaves, each being produced from the base of the *second* older leaf. The intrapetiolar stipule of any given leaf is initiated just after the appearance of the rudiment of the next younger leaf and at first it always incloses this rudiment.

By the time the fourth leaf rudiment has arisen, the outer tissues at the cut surface of the stem are usually ruptured and the young regenerated shoot has emerged. The first two leaves, which, as already mentioned, differ from the subsequent ones in that they arise from dedifferentiated cells of the original stem tissue, remain rather small, and differ in form from the subsequent leaves. The first leaf in transverse section is crescent-shaped (fig. 12). At an early stage in its history, its apex and lateral margins, by continued growth, come to inclose the second leaf (and later leaf rudiments) except at its dorsal side (fig. 8). The first leaf continues to grow at relatively the same rate as the second leaf and later leaf rudiments, until shortly after the young shoot has emerged, when its growth ceases. The first leaf rarely develops a blade, and it may be considered equivalent to the base only of one of the later leaves. The second leaf, although it develops a blade, as do the subsequent ones, differs from the latter in that its blade, which continues to grow for a short time, does not become differentiated into forked linear lobes. The third and all later leaves, however, do show such a differentiation (fig. 11). Hence the second leaf is a structure "transitional" between the first and third leaves.

The axis of the regenerated shoot always continues that of the original shoot and its plane of symmetry is also the same as that of the latter. The growth of the young shoot after it has emerged is rapid. Its stem, which continues to increase in radial diameter, soon attains that of the original stem and the juncture between the two becomes more and more difficult to distinguish, until finally there is left only a narrow ring or collar to indicate that regeneration has occurred.

As already stated, there is no stem growing point and each leaf arises from the base of the *second* older leaf. Therefore, the elongation of the stem axis is brought about by the growth and divisions of cells principally at the bases of the newly formed leaves. The vascular system of the young regenerated shoot begins differentiation by the time the third or fourth leaf rudiment has arisen. Within the central region of the meristematic tissue beneath the young leaf bases, a group (sometimes two or more groups) of tracheids is formed from the elongated cells of this region. These tracheids arise inde-

pendently of the original stem tracheids, which by this time have usually become disintegrated. The new tracheids continue to differentiate to a considerable distance within the bundle, toward the base of the stem. The cells within the young leaves, except those of the first, soon begin to elongate; and into the leaves bundles of tracheids extend from the meristematic tissue beneath the young leaf bases. There is neither a trace of a bundle in the first leaf (fig. 12) nor do tracheids extend in that direction from below. From the group of tracheids in the meristematic tissue, a bundle of tracheids extends into the second and third leaves at about the same time. The bundle of the second leaf, however, often arises independently of the group of tracheids. Shortly after the fourth leaf rudiment appears, the bundle which extends into it arises from that of the third leaf. The bundle of the fifth leaf, after it is formed, branches from that of the fourth. This method of bundle formation is the same in all subsequent leaves, so that a transverse section of the stem would show usually three bundles arranged in a crescent-shaped row, of which the concave side faces the dorsal side of the stem (the side toward the root).

When the rows of tracheids first appear in the bundle of the young leaf, they may at times extend at least half the length of the young leaf. But as growth proceeds, the further formation of tracheids gradually ceases. Consequently any transverse sections of a mature leaf, except at the base of the petiole, would not exhibit xylem elements. And even here, owing to an extensive stretching by the rapidly growing surrounding tissues, the only indications of their having been present may often be annular or spiral thickenings of those portions of the cells which have collapsed, accompanied by the pressing inward of the adjacent parenchyma cells of the thin parts of the walls between the rings or spiral thickenings. This stretching of the xylem elements also may occur in the rapidly growing region of the stem below the leaf bases, and frequently no xylem elements can be detected.

Branching of the young shoot frequently occurs and may take place when the young leaf rudiments are being formed at the time of regeneration (fig. 13). There are some indications that branching at this early stage is associated with the branching of the bundles of

the original stem. In such cases the first and second leaves appear to arise in the usual manner, and at the base of the first leaf the rudiment of the third leaf arises just as in the ordinary method of regeneration. Simultaneously with initiation of the first leaf or sometimes later, a leaf, equivalent to the first, is differentiated from the meristematic tissue at the side of the second leaf opposite the first in such a manner that the second leaf stands between and in the same plane with the two equivalent leaves. A third leaf arises at the base of each of the equivalent ones and each third leaf is inclosed at first by the intrapetiolar stipule of the second leaf (which is a dithecous one as shown at l_2 (fig. 13, *dl*). This gives rise to a type of dichotomy, and further growth of the two young shoots is similar to that of the ordinary branched shoots in this plant.

Regeneration from leaf

The leaf also shows a marked capacity for regeneration (fig. 1). When leaves are broken off from plants in their natural habitat, the detached parts are washed away and consequently their capacity to regenerate is easily overlooked. However, the laboratory method of culture shows that leaves can regenerate if cut off close to the stem. If they are cut off too far from the stem, they usually die.

A detached leaf produces at its proximal cut end a root, which continues the axis of the leaf. This root arises from the vascular bundle of the leaf in the same manner that a regenerated root arises at the basal cut end of the stem. On the other hand, if the cut is such as to leave part of the petiole (0.5 mm. or less) on the stem, a shoot often arises from the distal end of this. If the leaf is removed entirely, regeneration occurs from the stem at the point of detachment. A shoot, which continues the axis of the leaf, arises from the leaf bundle at the distal cut end in much the same manner that a shoot arises from a vascular bundle at the distal cut end of a stem.

Summary

1. *Podostemon ceratophyllum* has an unusual capacity for regeneration, which is possessed by each of its vegetative organs, and this regeneration can readily be observed in laboratory cultures.

2. If the distal end of a root is cut off, a new root growing point is

initiated back of the cut surface. The root cap is formed from the tissue between the meristem of the root growing point and the cut surface.

3. Occasionally cases of heteromorphism occur in root cuttings.

4. In contrast to known cases of regeneration of root tips in other forms, a new root growing point may be formed in *Podostemon ceratophyllum* regardless of how much of the distal end of the original root is cut off.

5. A root is also initiated at the proximal cut end of a stem, and it continues the axis of the latter. Its dorsal and ventral sides correspond to those of the stem.

6. A shoot is regenerated at the distal cut end of a stem. The first two leaves of the regenerated shoot arise from dedifferentiated cells within the stem bundle a short distance back of the cut surface. The regenerated shoot continues the axis and the dorsiventral symmetry of the original stem.

7. A detached leaf regenerates a root at its proximal end; the distal end of the petiole regenerates a shoot. In both cases the regenerated organ continues in line with the axis of the original organ.

8. When two or four roots are regenerated at the same cut end of a root, these may be laterally concrescent for 1 mm. to 10 cm. or more. Two shoot axes also may arise simultaneously at the distal end of a stem fragment. These are not concrescent.

The writer wishes to acknowledge indebtedness to Professor DUNCAN S. JOHNSON, under whose direction this investigation was carried out, for his helpful suggestions and valuable criticisms.

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THE CHROMOSOMES OF SPHAEROCARPOS CRISTATUS

CHARLES E. ALLEN

(WITH TWENTY-ONE FIGURES)

Introduction

In all species of *Sphaerocarpos* previously studied cytologically, the chromosome complement is essentially similar; the female having eight chromosomes one of which is a relatively very large X, the male having eight including a very small Y. This statement holds for *S. donnellii* (**1**, **2**), the American and European forms classed as *S. texanus* (**7**, **13**, **4**), and *S. michelii*, still called by European writers *S. terrestris* (**4**).

All these forms are obviously closely related; the gametophytes are practically indistinguishable, the only reliable diagnostic characters being those of the spores, which in all cases have areolate walls. Indeed, the specific distinctions within this group are much in need of further study.

LORBEER (**5**) has noted certain differences in the X-chromosome among the species just mentioned; most notable are the smaller relative size of this chromosome, and the presence in it of two hyaline regions instead of one, in a European race of *S. texanus*. On this basis he considers the European *S. texanus* (which he calls *S. europaeus*) specifically distinct from the American species. He has, however, overlooked the fact that the X-chromosome in WOLFSON'S (**13**) material of *S. texanus*, collected near Brussels, lacks the distinctive characters of that in "*S. europaeus*"; on the other hand, it resembles closely the X-chromosome found by SCHACKE (**8**), as well as by TINNEY (**12**), in American races of *S. texanus*.

Through the kindness of Dr. ANNETTA M. CARTER, dried material of *S. cristatus* Howe bearing mature sporophytes was received in November, 1934. It had been collected in El Dorado County, California, on April 15 previously. With the exception of two, whose spores showed the general characters of *S. texanus*, all the sporo-

phytes in this material agreed closely with HOWE's (3) original description of *S. cristatus*. Spores from ten of the *S. cristatus* sporophytes were sown, and some of the resultant sporelings, coming from spores of four sporophytes, were transplanted separately. Among the clones to which they gave rise are both females and males.

It is clear that, as emphasized by HOWE, *S. cristatus* is very distinct from the species of the *S. texanus* group. The spore wall bears sinuous ridges which only occasionally anastomose, instead of being regularly areolate; the spores separate early, whereas in the areolate-spored species they remain together in tetrads. Exceptions to the latter rule are certain races of *S. donnellii* whose spores separate in the course of maturation as shown by SILER (9)—not at maturity, as reported by previous investigators. In *S. cristatus*, too, the foot remains attached to the capsule when the latter is removed from the gametophyte; in the other species mentioned, the capsule separates from the imbedded foot. The gametophytes also differ in appearance; the thallus lobes of *S. cristatus* are more rigid and more uniformly entire-margined.

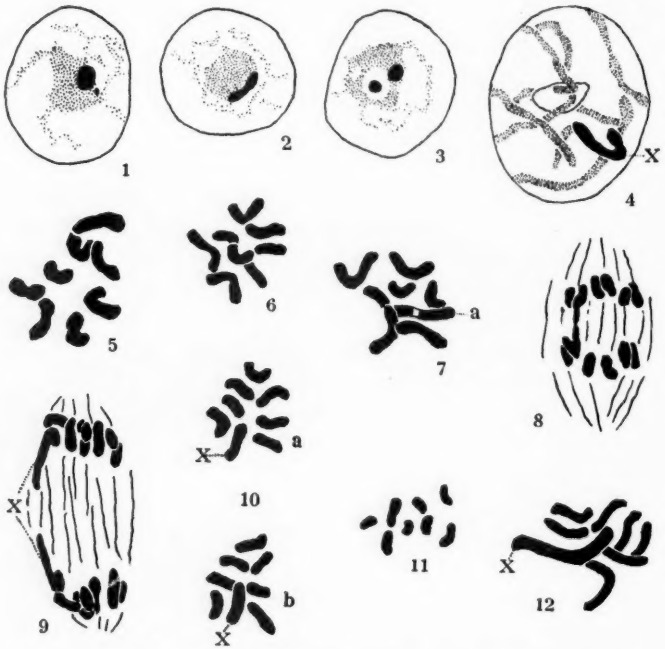
The distinctness of this species suggested a study of its chromosomes in comparison with those of the better known forms. Thallus tips of eight female and seven male clones were fixed and imbedded in paraffin by the usual method. Fixing fluids used were Carnoy's alcohol-acetic, Flemming's medium, the same diluted to four-fifths strength, and Sax's modification of Navashin's mixture. The three latter solutions were used alone, and also after dipping the material in the Carnoy fixative. Sections were stained with Heidenhain's iron-alum haematoxylin, except that some of the material fixed according to SAX's formula was stained by the crystal violet-iodine method as modified by SMITH (10). There is little to choose between preparations of material treated in these various ways; all have given usable and mutually corroborative figures.

Observations

FEMALE GAMETOPHYTE

In a polar view of an equatorial plate in a female plant of *S. cristatus* (figs. 6, 7), eight chromosomes are visible. The number is the same as in *S. donnellii*, but none of the eight is distinguished from

the others as is the X-chromosome in the female of the latter species (fig. 12) by its markedly greater length and thickness. Instead, all the members of the female chromosome complement of *S. cristatus* are approximately alike in diameter, although differing in



FIGS. 1–12.—Figs. 1–11, *Sphaerocarpos cristatus* ♀: figs. 1–3, resting nuclei, each showing one or two heteropycnotic bodies; fig. 4, prophase nucleus, the X-chromosome heteropycnotic; fig. 5, very late prophase chromosome group; figs. 6, 7, polar views of equatorial plates; figs. 8, 9, anaphases seen laterally; fig. 10, upper (*a*) and lower (*b*) sister anaphase chromosome groups in polar view; fig. 11, anaphase group in polar view. (Figs. 1, 4, 6, 7 from involucral cells; others from thallus cells.) Fig. 12, *S. donnellii* ♀: polar view of equatorial plate (involucral cell). All × c. 3600.

length. Their small size and the foreshortening to which some are inevitably subjected render statements as to comparative length necessarily tentative. But from a study of such plates as that shown in figure 7, in which all the chromosomes lie approximately parallel

to the plane of the section, it appears that there may be three relatively long elements, two of medium length, and three short.

Spindle fiber attachments of the short chromosomes, and possibly of one of the long ones, are median; those of the other chromosomes are submedian. The fiber attachment points are commonly marked by constrictions, as various of the figures show; very rarely the constriction region appears achromatic (chromosome *a*, fig. 7).

At the equatorial plate stage the chromosomes invariably overlap more or less, especially by their ends. In studying even such groups as those shown in figures 6 and 7, which are among the most favorable found, a determination of the chromosome number involves conclusions based upon relative depths of focus. In a very late prophase (fig. 5), however, or still better in polar views of anaphases (figs. 10, 11), the chromosomes are often entirely separate from one another and the count of eight can be made with objective certainty.

Evidently at the equatorial plate stage an X-chromosome cannot be distinguished, although comparison with the chromosome complement of the male makes it certain that one of the chromosomes of the female is an X. There are, however, peculiarities of behavior which at certain stages make possible a reasonably confident recognition of this element.

In regions of the female plant, such as the growing tip, where cells are small and divisions succeed one another at relatively short intervals,[1] if the preparation is sufficiently destained, one small dark-staining body is commonly seen in a resting nucleus. This body, rounded (fig. 1) or elongated (fig. 2), is regularly in contact with the more lightly stained central mass consisting of nucleolus and adherent chromosome substance. Less often two smaller heteropycnotic bodies are present (fig. 3). The condition is precisely similar to that described by TINNEY (**11, 12**) as occurring in the females of *S. donnellii* and *S. texanus*. In both the latter species the heteropycnotic body has been shown to be a part of the X-chromosome.

[1] Some writers speak of such regions as meristematic. But it must be remembered that all or most of the cells of the thallus of *Sphaerocarpos*, although increasing in size, remain for a long time essentially meristematic, in the sense of being undifferentiated and capable of division. The possibility of the division of relatively old cells is illustrated by the very frequent occurrence of regeneration from various parts of the thallus (**6**).

Although for obvious reasons this cannot be demonstrated to be the case in *S. cristatus*, there can be little doubt that in this species also the heteropycnotic body represents either a part or the whole of an X-chromosome.

LORBEER (**5**) apparently considers that there are regularly two heteropycnotic bodies in resting nuclei of *S. donnellii*, representing respectively the two arms of the X-chromosome. Our preparations, however, show clearly that there is most frequently only one such body.

It cannot be too frequently emphasized that "heteropycnosis" is a relative appearance, affected by the condition of the cell, particularly, as TINNEY (**11**) has shown, by the length of the interkinetic period; probably affected also by the fixation, and certainly by the staining procedure. It is possible in material destained to different degrees to find nuclei with no stain whatever, others with one, two, three, or more stained bodies, and still others whose chromosomal and nucleolar substances are uniformly and deeply stained. One expecting any particular number of heteropycnotic bodies might find evidence to fit his preconception. It is only when a very large proportion of the nuclei in satisfactorily stained material shows a like condition that significant conclusions can be drawn regarding heteropycnotic phenomena.

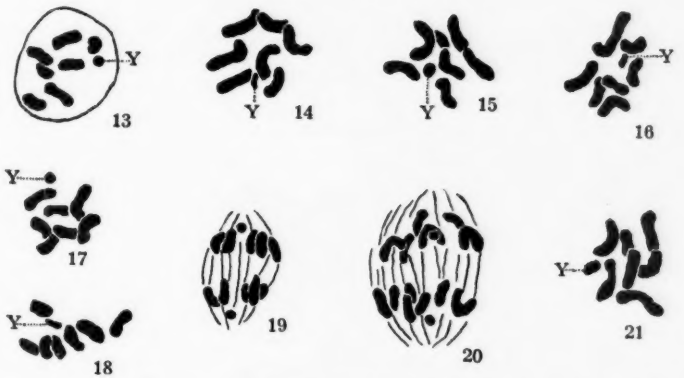
In median prophase stages (fig. 4), one chromosome is sometimes seen to be more condensed and much more deeply stained than the others. This condition again is characteristic of the X-chromosome at corresponding stages in *S. donnellii* and *S. texanus* (**11, 12**).

In lateral views of anaphases (figs. 8, 9), one chromosome, apparently one of the longer ones, is regularly seen to lag somewhat behind the others. This behavior is that typical of the X-chromosome in *S. donnellii* (**2**). Sometimes, also, in a polar view of an anaphase the lagging chromosome can be recognized. In figure 10 *a*, the chromosome marked X is the member of the upper anaphase group one of whose ends extends farthest downward, and the similarly indicated chromosome in figure 10 *b* is the member of the lower group which extends farthest upward. Hence, although the X-chromosome is not clearly distinguished by its size, as in the species of *Sphaerocarpos* previously studied, what may confidently be considered to

be this element is identifiable by a heteropycnotic condition in resting and prophase stages and by a delayed poleward movement in the anaphases.

Male gametophyte

The chromosome number in a male plant of *S. cristatus* is likewise eight, as seen in late prophases (fig. 13) and in polar views of equatorial plates (figs. 14–17) and of anaphases (fig. 18). One of the eight is very small, appearing either approximately spherical (figs.



Figs. 13–21.—Figs. 13–20, *Sphaerocarpos cristatus* ♂: fig. 13, prophase nucleus, chromosomes organized; figs. 14–17, polar views of equatorial plates; fig. 18, anaphase group in polar view; figs. 19, 20, anaphases seen laterally. (Figs. 13, 15, 16 from involucral cells; fig. 17 from an androgonium; others from thallus cells.) Fig. 21, *S. donnellii* ♂: polar view of androgonial equatorial plate. All × c. 3600.

13, 15, 17) or somewhat elongated (figs. 14, 16, 18). Comparison with the chromosome group of *S. donnellii* male (fig. 21) shows a close similarity throughout. The very small chromosome present in the male and not in the female of *S. cristatus* evidently corresponds to the Y-chromosome of *S. donnellii*.

So far as the difficulties in the way of formulating a judgment on this point permit, it appears that there are two relatively long chromosomes in the male (see especially figure 16) instead of three as in the female. This, if correct, would support the conclusion that the X of the female gametophyte is one of the longer chromosomes.

No evidence has been found of the regular presence of a heteropycnotic body in resting or prophase nuclei of the male plant. In this respect, too, *S. cristatus* resembles *S. donnellii* as described by TINNEY (11). LORBEER'S (5) statement that the Y-chromosome of *S. donnellii* is heteropycnotic is not borne out by our studies of that species.

Nor does it appear that the Y-chromosome lags behind the others during the anaphases. Sometimes, indeed, as in the anaphase shown in figure 19, it appears that the Y may precede the other chromosomes to the pole. But such appearances as that of figure 20 indicate that its behavior in this regard is variable.

While, therefore, the male chromosome complement of *S. cristatus* is closely similar to that of *S. donnellii*, and while the chromosome number is the same in both species, the marked difference in the relative size of the X-chromosome parallels the external differences which have already suggested the placing of these species in distinct subgeneric groups.

Summary

1. The chromosome number in both female and male gametophytes of *Sphaerocarpos cristatus* is eight.

2. The chromosome complement of the female includes no element that is conspicuously distinguished by its size. One chromosome (or part of one), however, is heteropycnotic in resting and prophase stages; and one chromosome lags behind the others in the anaphasic movement to the poles. These peculiarities, common to the large X-chromosome of other species of the genus, make it reasonably certain that the chromosome so distinguished (probably one of the longer ones) is the X.

3. The chromosome complement of the male includes a very small Y. This is not heteropycnotic.

4. *S. cristatus* is set apart by its external characters from other species (*S. donnellii*, *S. texanus*, and *S. michelii*) which have been studied cytologically. The much smaller size of the X-chromosome in *S. cristatus* as compared with the other species mentioned is an additional distinguishing mark.

The work whose results are here reported was carried on with the aid of grants from the Wisconsin Alumni Research Foundation and with the assistance of Dr. FRED W. TINNEY and Miss DOROTHY C. BAUCH. Sections were made by Miss ELEANOR H. ARTMAN under an FERA appointment.

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EFFECT OF AUXINS FROM SOME GREEN ALGAE UPON PHYTOPHTHORA CACTORUM

LEON H. LEONIAN[1]

(WITH TWO FIGURES)

There are many references in the literature concerning the auxins and hormones produced by bacteria, fungi, and phanerogams. In so far as the writer knows, however, no work has been done with algae.

Through the courtesy of Dr. FLORENCE E. MEIER, four pure cultures of unicellular green algae were obtained as follows, *Chlorella viscosa*, *Coccomyxa simplex*, *Oocystis naegelii*, and *Scenedesmus flavescens*. Originally these cultures were secured with the specific purpose of testing upon them the growth-promoting substances extracted from mature garden peas. These substances are a controlling factor in the growth of a great many filamentous fungi, while in case of others they produce a remarkable stimulation of both growth and reproduction.

All four of these algae make a moderately good growth on a medium consisting of 5 gm. of dextrose, 1 gm. of potassium nitrate, 0.5 gm. of dihydrogen potassium phosphate, 0.25 gm. of magnesium sulphate, and 20 gm. of agar-agar in 1000 cc. of distilled water. When, however, 0.05 per cent of pea extract (obtained by extracting ground peas with 95 per cent of alcohol in soxhlet) was added to this solution, the algal colonies grew much more rapidly (fig. 1). Ash of garden peas exerted no such effect. *Chlorella viscosa* and *Coccomyxa simplex* eventually lose their bright green color on agar cultures containing no pea extract and become pale yellow, with the green color confined to a small area around the original inoculum. Under the microscope the cells appear almost colorless and their protoplasm seems granular and thin. In cultures containing the pea extract, however, the colonies retain their color throughout their life (five months), and the individual cells appear bright green, plump, and

[1] Published with the approval of the Director of West Virginia Agricultural Experiment Station, as Scientific Paper no. 155.

full of protoplasm. While in case of *Ooocystis naegelii* and *Scendesmus flavescens* there is some stimulation of growth and especially of color, it is not so pronounced.

When transferred to the nutrient solution of the foregoing composition, *Phytophthora cactorum* fails to make any growth whatever, while excellent growth follows upon the addition of a trace of pea extract. Since all four of the algae can grow in this solution, one may safely assume that they are capable of manufacturing their own growth-promoting substances. Yet the slower growth and the loss of

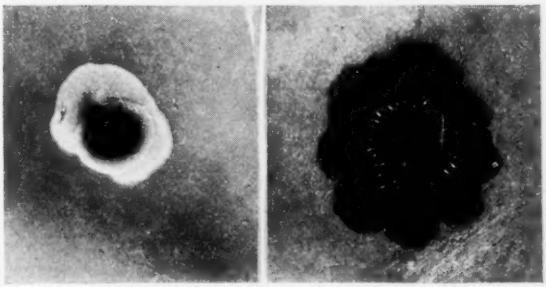

FIG. 1.—Effect of growth-promoting substances upon *Oocystis naegelii*. Left (check), no growth-promoting substances present in the nutrient agar. Right, a trace of growth-promoting substances from the garden peas added to the agar.

color seem to show that the growth-promoting substances manufactured by the algae themselves, and those extracted from garden peas, may either be unlike chemically or may differ quantitatively.

The next procedure was to test the effect of the algal substances upon the development of *Phytophthora cactorum*. The following method was used: The algae were grown in flasks containing 200 cc. of the nutrient solution already mentioned but having received no pea extract. After 30 days at room temperature and in diffused light these pure cultures were filtered aseptically through two thicknesses of sterilized, fine filter paper. Ten cc. of the filtrate was poured into preparation dishes of 25 cc. capacity and inoculated with *P. cactorum*. The check solution in which no algae had been grown was treated similarly. There was no growth in the check, while excellent growth resulted in the filtrates of the cultures of *Coccomyxa simplex*

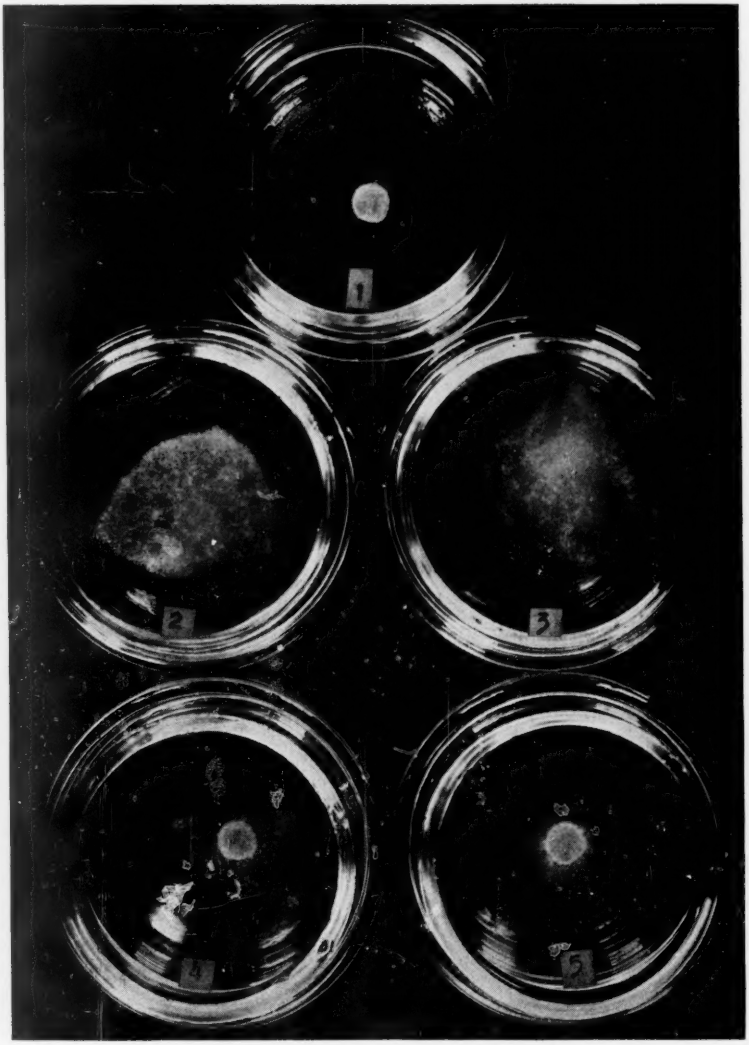

FIG. 2.—Effect of growth-promoting substances from some unicellular algae upon the growth of *Phytophthora cactorum*. 1 (check), no algae grown in the nutrient solution; 2, filtrate of *Oocystis naegelii;* 3, filtrate of *Coccomyxa simplex;* 4, filtrate of *Chlorella viscosa;* 5, filtrate of *Scenedesmus flavescens*.

and *Oocystis naegelii;* the filtrates of *Chlorella viscosa* and *Scenedesmus flavescens* induced only a fair growth (fig. 2).

In addition to auxin content, the algal filtrates were tested also for sexuality-promoting substances. *Phytophthora cactorum* produces an abundance of oogonia and antheridia on suitable media, while no such bodies are formed on others. If 0.2 per cent of proteose peptone be added to the foregoing nutrient solution, *P. cactorum* will grow very well, but without forming any reproduction bodies. Thus it is possible to obtain a well nourished but sterile mycelium which can be subjected to the action of various environmental factors. Four days after the transfer of the inoculum, the fungus produces a colony large enough to be washed in distilled water and be transferred to any de-

TABLE I

EFFECT OF ALGAL FILTRATES UPON P. CACTORUM

FILTRATE	SPORANGIA	OOGONIA
Coccomyxa simplex	Abundant	Abundant
Oocystis naegelii	Abundant	Abundant
Chlorella viscosa	Very abundant	Few
Scenedesmus flavescens	Abundant	Few
Check (nutrient solution)	None*	None

* If allowed to remain in the nutrient solution eight days, the mycelium forms sporangia but no oogonia.

sired solution. The mycelial colonies of *P. cactorum* were washed thoroughly in sterile distilled water and then transferred to a small amount of the algal filtrate (2 cc. in preparation dishes of 25 cc. capacity); when after three days at 20° C. they were examined under the microscope, it was observed that both sporangia and oogonia had formed (table I).

In connection with our project on the isolation and identification of growth and sexuality promoting substances, Dr. VIRGIL G. LILLY and the writer have tested a great many substances, green algae included. *Oocystis naegelii* was first grown in a solution consisting of the essential salts and glucose; a month later the algal cells were filtered, washed with distilled water and extracted. The alcoholic extract proved to be rich in both growth and sexuality promoting factors for *Phytophthora cactorum*, while the ether extract failed to

induce any growth but was found to be very rich in sexuality promoting factors. This is in general agreement with results which we have obtained from other substances.

As the extracts from the algal cells proved to be much richer than the filtrate in their ability to induce growth and sexual reproduction, it follows that the factors responsible for this are manufactured by the algae themselves instead of being due to any chemical changes brought about in the medium by the growing cells of *Oocystis naegelii*.

It is comparatively safe to conclude that these algae produce sexuality-promoting substances in addition to auxins, and that the quantity of these substances diffused into the solution varies with different species of algae in so far as the response of *P. cactorum* is concerned. Whether these substances are diffused through the plasma membrane of the living algal cells or whether they are liberated upon the death and subsequent lysis of the cell is not yet clear. The writer (2) has demonstrated that uninjured roots of corn seedlings do not give off auxins into the solution in which the roots are kept growing, while from the proximal part of a detached root considerable quantities of auxins diffuse out into the medium. Filamentous fungi, on the other hand, give off auxins into the medium without having their mycelium subjected to any apparent injury. Presumably the auxins diffuse through the uninjured plasma membrane. An extensive series of dialysis experiments by the writer and by his collaborator, Dr. LILLY, have shown that auxins from various sources readily pass through parchment paper and through collodion sacs. Furthermore, it is well known that certain pathogenic bacteria such as *Hemophilus influenzae*, which grow in artificial cultures with great difficulty, produce a luxuriant growth in the immediate neighborhood of a colony of some staphylococcus because of the growth-promoting substances which diffuse into the agar from the colony of the latter organism. Such data tend to indicate that at least in certain cryptogamic organisms the auxins may diffuse out into the medium through uninjured plasma membrane.

The foregoing results show that the algal and fungal flora of the soil may possibly exert some influence upon green plants as well as upon cryptogamic inhabitants of the soil. ITO and SHIMADA (1) and

SHIMADA (3) have shown that the addition of a filtrate of *Gibberella fujikuroi* to nutrient solutions induces a remarkable stimulation of growth in seedlings of rice, wheat, barley, Indian corn, Azuki bean, and soy bean. This stimulation is apparently caused by the auxins given off by the fungus. It is possible that similar results may occur under field conditions by the association of suitable cryptogamic flora and phanerogamic plants.

The data obtained in this work also shed some light upon the relationship between the fungi and algae which grow together to form lichens. It is probable that the lichen fungi utilize not only carbohydrates furnished by the algae, but growth substances as well, and that their compatibility and the subsequent associations may not be controlled so much by the nature of carbohydrates as by the nature, the quantity, and the availability of the growth- and reproduction-inducing substances.

Summary

1. Four pure cultures of unicellular green algae, *Chlorella viscosa*, *Coccomyxa simplex*, *Oocystis naegelii*, and *Scenedesmus flavescens*, have been used. Especially the first two show a remarkable stimulation under the action of growth-promoting substances extracted from garden peas. These algae also retain their green color in the presence of these substances, while in their absence the color largely fades into a yellowish tone.

2. All four of the algae form both growth and sexuality-promoting factors for *Phytophthora cactorum*.

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CHROMOSOME NUMBERS AND ELECTROPHORESIS OF LATEX IN ASCLEPIAS

LAURENCE S. MOYER[1]

(WITH SEVEN FIGURES)

Introduction

Evidence has recently been presented (**8**) to show that the surfaces of the latex particles of *Euphorbia* species are constant in composition, depending, within broad limits, only on the species and not upon the environment. This specific character of the surfaces is exhibited in the isoelectric points, wetting properties (**10**), and the shapes of the electrophoretic mobility curves when plotted against pH (**6**). When these mobility curves were classified according to their shapes, they fell into groups in correspondence with the systematic groupings of the species. Furthermore, in the section Poinsettia, the presence of an aberrant member, *Euphorbia heterophylla*, was first indicated by its latex behavior and then was shown to be a polyploid from chromosome counts (**7**). All these observations were confined to a single genus. To test the generality of these phenomena, determinations of chromosome numbers and latex mobility curves have been made on species of *Asclepias*.

The investigations were limited by the paucity of latex in certain species and by the number of available species growing within convenient reach of the laboratory. Although the number of species reported here is not so great as could have been desired, enough data are available to demonstrate that the use of latex as a taxonomic tool need not be limited to *Euphorbia*.

Methods

The technique of the chromosome counts was the same as previously described (**7**). All root tips were cut at 8μ. For the electrophoretic measurements, latex was in all cases suspended in the buffer solutions in the field or greenhouse; all determinations were performed within an hour after suspension, usually within ten minutes. Acetic acid-sodium acetate buffers of constant ionic strength ($\mu =$

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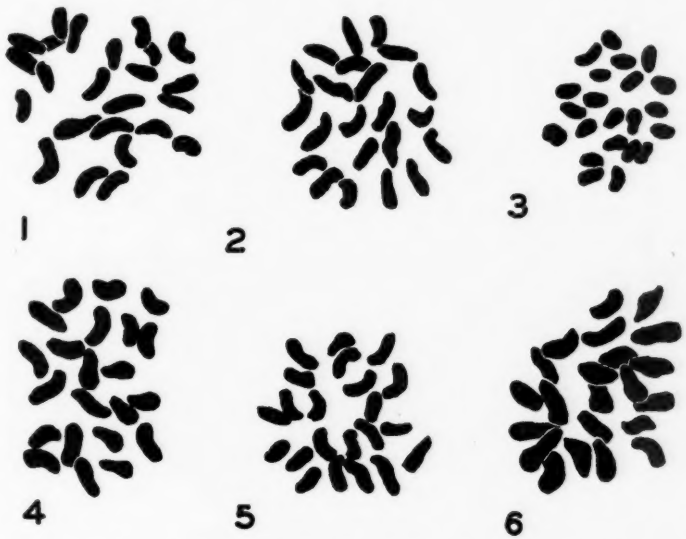
1/50) were used. The specific resistivity of these buffers is nearly constant, making them superior to mixtures of constant molarity (as used before, **6**) for electrophoretic work. The preparation of such buffers has been described (**9**). The technique of electrophoresis was the same as before (**6, 9**). Mobilities are given in μ/sec./volt./cm., corrected to 25° C.



FIGS. 1–6.—Somatic metaphase plates of *Asclepias:* fig. 1, *A. syriaca* 2n=24; fig. 2, *A. incarnata* 2n=22; fig. 3, *A. curassavica* 2n=22; fig. 4, *A. salicifolia* 2n=22; fig. 5, *A. latifolia* 2n=22; fig. 6, *A. tuberosa* var. *sulfurea* 2n=22. ×4000.

Results

The results of the chromosome count are depicted in figures 1–6. *Asclepias curassavica* L., *A. salicifolia* Lodd., *A. incarnata* L., *A. latifolia* Rafin., and *A. tuberosa* var. *sulfurea* L. have 22 somatic chromosomes whereas *A. syriaca* L. (=*A. cornuti* Decaisne) has 24.[2] The morphology of the chromosomes is noticeably different among the different species although the chromosomes are small and short.

The electrophoretic curves of the latex particles are shown in figure 7. The latex particles seem slightly larger than those of

[2] These counts have been checked by Mr. A. O. DAHL in addition to the writer.

Euphorbia and exhibit more tendency to cohere. The chromosome numbers and isoelectric points are summarized in table I. No electrophoretic data on *A. latifolia* or *A. tuberosa* var. *sulfurea* are avail-

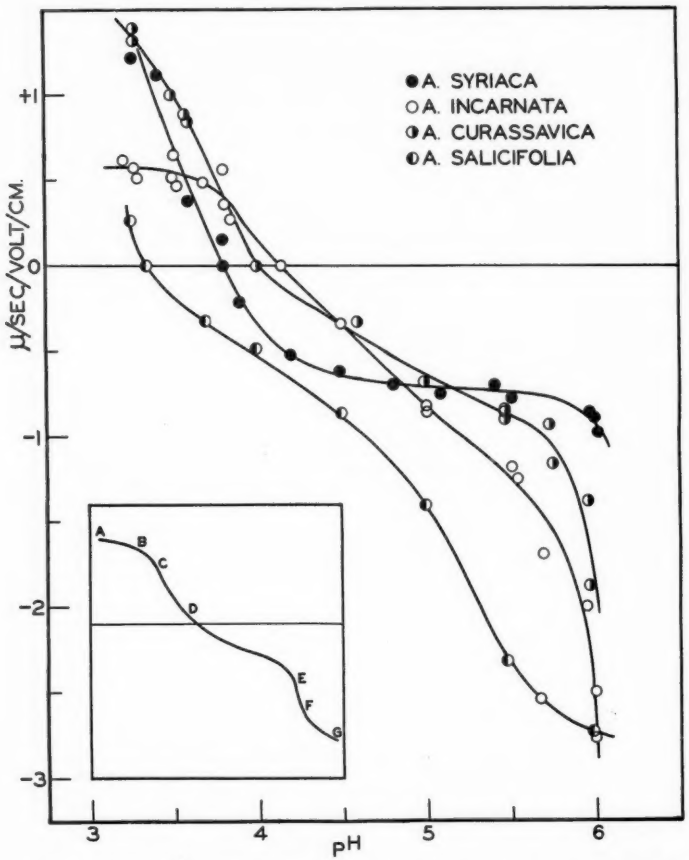


FIG. 7.—Electrophoretic mobility curves of latex particles from species of *Asclepias*. Inset shows hypothetical curve (over a wide pH range) approximated by the various species.

able, owing to the scarcity of latex in these species. As may be seen, the extra chromosome of *A. syriaca* causes no sharp change in its isoelectric point or curve shape from the rest. The isoelectric points and

shapes of the mobility curves (fig. 7) of *A. syriaca*, *A. incarnata*, and *A. curassavica* over the range from pH 3 to 6 are rather closely allied. *E. salicifolia* is the most divergent species of the lot. Results were sometimes more variable than those obtained with *Euphorbia* but with proper care data could be checked. The present isoelectric pH values for *A. syriaca* and *A. curassavica* agree well with results (pH 3.9 and 4.1 respectively) obtained under different conditions with plants from a different source (**10**). This slight divergence of 0.1 pH unit may be ascribed to differences in the concentrations of the

TABLE I

CHARACTERISTICS OF ASCLEPIAS SPECIES

SPECIES	LATEX ISOELECTRIC POINT (pH)	CHROMOSOME NUMBER (2n)	POSITION ON HYPOTHETICAL CURVE
A. incarnata	4.1	22	A–F
A. curassavica	4.0	22	B–F
A. syriaca	3.8	24	C–E
A. salicifolia	3.4	22	D–G
A. latifolia	.....	22	.....
A. tuberosa sulfurea	.....	22	.....

buffer systems. Plants tapped after fruiting sometimes yielded divergent and variable results; such plants were not in active growth and appeared much darker in leaf color. Only data from young healthy plants are presented here.

Discussion

These curves are limited in extent by the range of the buffer (pH3–6). Introduction of another buffer introduces complications which would invalidate comparisons. Since the range is so narrow, only a part of the total curve for each species is represented. From this, however, we can construct a hypothetical curve with which all of the species here reported agree in shape for the portion over which they extend. The portions of the hypothetical curve fitted by the various species are shown in table I and figure 7 (inset). It will be noted that as the isoelectric point descends on the pH scale the curve is merely moved over one more letter in each species. Hence, the curve of each species would represent the portion of this hypothetical curve (shifting with the isoelectric point) confined between the limits of the

measurements. Minor differences in shape do occur but they seem to be secondary in nature.

By the use of the Mudd interfacial technique it was shown (10) that *A. syriaca* and *A. curassavica* possess a hydrophilic surface which strongly resists wetting by oil. The position of the isoelectric points and the shapes of the mobility curves for these species indicate that proteins are present on the latex particle surfaces (1), in complete agreement with the wetting data. The surfaces are undoubtedly complex in nature. No wetting determinations have been made on *A. salicifolia* and *A. incarnata* latex.

STEVENS (11), GAGER (5), and FINN (2) also report 24 chromosomes as the diploid number for *A. syriaca*. FRYE (3, 4) states that the haploid counts for *A. tuberosa* L. and *A. sullivantii* Engelm. are about 5. He also states that gametophytic material of *A. verticillata* shows about 8 chromosomes but he was unable to give more definite figures. No other species of *Asclepias* appear to have been counted. The lack of a marked variation in the shape of the curve of the aneuploid species (*A. syriaca*) indicates that genes controlling the surfaces of its latex particles are not located in the extra chromosome.

Since so few species have been investigated, an attempt to show any sort of phylogenetic arrangement on the basis of either chromosomes or latex curves would seem premature. The data indicate, however, that species relationships are shown by the electrophoretic curves of the latex particles of *Asclepias* as well as in the case of *Euphorbia*.

Summary

1. The electrophoretic mobility curves for latex particles of *Asclepias syriaca*, *A. curassavica*, *A. incarnata*, and *A. salicifolia* have been determined.

2. The chromosomes from the root tip cells of these species and also *A. tuberosa* var. *sulfurea* and *A. latifolia* have been counted. *A. syriaca* has 24 somatic chromosomes while the rest of the species have 22.

3. The presence of this extra chromosome in *A. syriaca* is not shown by the electrophoretic curve, indicating that genes for the latex particle surface are not located in this chromosome.

4. The isoelectric points of *Asclepias* appear to be constant and

dependent only on the species, within broad environmental conditions.

5. The surfaces of the latex particles are, at least in part, coated with proteins.

6. Species relationships are shown by the electrophoretic curves and isoelectric points of the latex particles of *Asclepias* as well as of *Euphorbia*. All of the species in the present work are characterized by the same type of mobility curve.

The writer wishes to thank the Botany Department of this University for extending the facilities of its equipment during the preparation of the cytological material. Thanks are especially due to Professor R. A. GORTNER, in whose laboratory the investigations were performed, for his kind help and suggestions.

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CURRENT LITERATURE

Invisible Radiations of Organisms. By OTTO RAHN. Berlin: Gebrüder Borntraeger, 1936. Pp. x+215.

About 14 years ago GURWITSCH published his first papers dealing with the causes of cell division, and the existence of radiations emanating from the rapidly dividing cells of root tips, which radiations excite other root tips to increased cell division, or excite yeast cells to more rapid budding. These phenomena soon came to be known as mitogenetic radiations. In the period following these original contributions of GURWITSCH, many investigators have worked in this field and there has been controversy as to the reality of the mitogenetic radiation phenomena. Many European investigators have supported GURWITSCH's observations and interpretations, while some of the American workers have been conservative and skeptical.

The results of the work done since 1922 are brought together in summary fashion as a monograph (Protoplasma Monographien, vol. IX), by OTTO RAHN, Cornell bacteriologist, who believes in the reality of the phenomena, although it appears that he has had rather moderate experience in detecting them in his own work. In the foreword he claims that invisible radiations from organisms are not at all strange, and that if GURWITSCH had not discovered them some years ago, they would now be predicted from the results of physicochemical investigations.

The introductory chapters, contributed by SIDNEY W. BARNES, will be valuable to students of biology because they contain a clear and concise explanation of radiation physics, wave theory, quantum theory, dispersion, intensity measurements of visible and invisible radiations, physical, chemical, and secondary sources of radiation, and the effects of invisible radiations upon chemical reactions and upon living cells.

The mitogenetic radiations are given extensive treatment in chapters IV–VI. Chapter IV considers the methods used for detection of biological radiation; chapter V discusses the special characteristics of these radiations; and chapter VI makes an analysis of the mitogenetic effect.

The last chapter is devoted to the significance of biological radiations in biology, medicine, and agriculture. In connection with blood and carcinoma radiations, medicine may find considerable aid in diagnostic and other problems. At the close of chapter VII the author presents the "outlook" for this field, and also gives a brief summarizing statement of the various chapters. There are author and subject indices, the former incomplete but extensive enough for the purposes of the monograph. The author is convinced from his own experiences

as well as from a study of the literature that mitogenetic radiation is a fact. He has undoubtedly presented the subject in its best light. If the skeptics and the enthusiasts could work together for a while the reasons for controversy would probably soon be eliminated. RAHN claims that errors and misunderstandings rest on both sides, and he has tried to point out some of the sources of disagreements. It does not seem unreasonable to ask those who believe in mitogenetic phenomena to define their methods and conditions clearly enough that the skeptically inclined may follow them. One of the difficulties seems to be that failures to obtain the phenomena are explained away by the believers as due to the use of material in the "wrong physiological state," or by saying that "unknown factors" have interfered. The monograph is interestingly written, and will prove valuable in stimulating work toward the settlement of the controversies in this field.—C. A. SHULL.

The Theoretical Significance of Vernalization. By N. A. MAXIMOV. Imp. Bur. Plant Genetics, Herb. Publ. Series Bull. 16, 1934. Pp. 14.

Vernalization and Phasic Development of Plants. Imp. Bur. Plant Genetics Bull. 17, 1935. Pp. 155.

Initiated as a practical agricultural method of accelerating the development of economic plants and solving such problems as the transformation of winter wheat into spring wheat, vernalization has involved such a control of the phenomena of growth and development of living organisms that it has attracted the attention of plant physiologists throughout the world. Since much of the experimental work in this field has been carried on by Russian scientists, among whom LYSENKO has been a leader, it is fitting that its theoretical significance should be discussed by a leading Russian plant physiologist. MAXIMOV has pointed out that much of the recent investigation is a continuation and extension of the work of KLEBS, who proved that the life processes of plants, including reproduction, are to a great extent subject to the control of external conditions, and that by modifying these conditions man may change the internal state of many plants and so alter such phases of development as flowering and seed production.

Following to a large extent similar principles, LYSENKO's hypothesis of vernalization is largely based on the method of subjecting slowly germinating seeds of winter wheat to a temperature little above 0° C. for periods of 10 to 60 days. The seed is then sown in suitable soil, at normal spring temperatures, and the resulting plants behave like spring wheat and produce a crop during the same summer. His theoretical concepts are that growth and development are not identical phenomena, that the entire process of the development of an annual seed plant consists of individual steps or stages, that these stages always proceed in a strict sequence and a subsequent stage cannot set in until the preceding stage has been completed, and that different stages of the same plant require different external conditions for their completion.

These concepts MAXIMOV has examined in detail and discussed their strong and weak points. He regards the hypotheses as well founded but probably subject to modification upon further experimentation.

In a more recent bulletin from the same source, an elaboration of LYSENKO's theories is presented, together with summaries of the results obtained by many other scientists who have investigated vernalization in a considerable number of plants. The idea that development consists of a series of stages has been elaborated and the "thermo-stage" and the "photo-stage" are discussed, the latter involving a somewhat different interpretation of "short-day plants." A third stage is less clearly differentiated and modifications of LYSENKO's theory by other investigators are given. It is evident that vernalization and its modifications have been extensively investigated in the Soviet Union. As the results of these investigations have been published almost exclusively in Russian, the Imperial Bureaux of Plant Genetics has rendered the scientists of Great Britain and America a great service by making the results available in English. The investigations of the problems related to vernalization in other countries have been summarized and brought down to date and a bibliography of nearly one hundred titles is appended.—G. D. FULLER.

The Garden Encyclopedia. Edited by E. L. D. SEYMOUR. New York: Wm. H. Wise & Co., 1936. Pp. x+1300. Illustrated.

Although rather brief encyclopedias on horticulture are already available, this newcomer should find a place among the books of everyone interested in that subject. The variety of subjects treated is widely comprehensive, but each is discussed in sufficient detail really to be useful to the reader. The botanical information given is accurate, concise, and helpful, but with no attempt at being exhaustive. The horticultural suggestions and directions are clear cut and as definite as can be expected, considering the wide variety of conditions under which various forms may be grown. The text is well illustrated with halftones and effective line drawings. Tabular summaries on some subjects are very useful.—E. J. KRAUS.

The Biochemistry of the Lipids. By HENRY B. BULL. Division of Biochemistry, University Farm, St. Paul, Minn.

In this mimeographed text the chemistry of the important groups of fatty acids, soaps, alcohols, sterols, fats, oils, phospholipids, and glycolipids has been ably summarized and excellently organized into a readable text. Tables of properties, graphs, and graphic formulae have been used so freely that the book is valuable for reference to this insufficiently known group of substances. Numerous references to the physiology of the lipids should be especially valuable to the plant physiologist.—R. B. HARVEY.

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